LABORATORY STUDIES IN ZOOLOGY

H. D. REED AND B. P. YOUNG

Laboratory Studies in Zoology. H. D Reed and B. P. Young. 1930. viii plus 121 pp. McGraw-Hill Book Company.

This laboratory manual has grown out of the elementary course in zoology at Cornell. Approximately half the book is devoted to outlines for the study of the frog. Keys are given for student identification of Protozoa and for "the larger groups of animals." This adds another to the distinctly teachable laboratory outlines in elementary zoology for the increasing number of teachers who dislike to "roll-their-own."

—W. C. Allee.

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PREFACE

The laboratory outlines here presented have been fashioned out of experience with students in the laboratory, extending over a considerable period of years. An attempt has been made to discover the most serious difficulties encountered by elementary students and to outline the procedure accordingly. An attempt has also been made to incorporate the quality of flexibility as regards detail of methods. Even though several instructors are engaged in conducting different laboratory sections in the same course, better results are obtained when each instructor is free to employ his own details of method, to act upon his own initiative, and to give expression to his own personality. Questions have been almost entirely eliminated, in the belief that questions formulated by the instructor would be more effective in stimulating the student to *see* and *think*.

The order in which the several studies are arranged need not necessarily be followed. The authors have found, however, that a beginning student when studying a series of animals, in whatever order they may be arranged, fails to grasp the significance of his studies as constituting a connected whole. A beginning study of a higher animal type obviates difficulties of this sort. The introductory study of the frog, it should be emphasized, is not outlined for the purpose of learning about the frog as such. It affords a means of inculcating the principles of bodily organization, the services rendered by bodily components, the nature of protoplasm, and the nature and importance of the cell as a vital unit; and finally it affords a means of building up a concept of the organism as a totality and superior entity. Incidentally, a knowledge of all of the fundamental biological principles which a beginning student can master may be gained in such a study. Following this a study of the animal types may be made more significant.

Should it appear that too many types and too many details are included, the aims of the teacher may be taken as the best guide in omitting parts or even whole studies. Added interest may be aroused by the display of living animals, demonstrations,

and models, according to the facilities of the laboratory and the ideas of the instructor.

It is assumed that the instructor has a much broader and more detailed knowledge of the subject than is represented in these outlines.

The authors wish to acknowledge their indebtedness to Dr. Mary J. Fisher, Dr. A. Grace Mekeel, and the Misses Eleanor C. McMullen and Lillian A. Phelps, who have used these outlines during the building period and have offered valuable suggestions and criticisms. Grateful acknowledgment is also made of the efforts and success of Miss Mary Mekeel in producing the illustrations.

H. D. R.
B. P. Y.

Ithaca, N. Y.
July, 1930.

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LABORATORY STUDIES IN ZOOLOGY

INTRODUCTION

GENERAL DIRECTIONS

Supplies, Textbooks, and Instruments.—Students in the course should provide themselves with the following:

1. A copy of the textbook chosen for the course.
2. Laboratory manual for elementary zoology.
3. A heavy grade of drawing paper, 8 by $10\frac{1}{2}$ inches.
4. Large Manila envelopes for laboratory records.
5. Note paper and covers 8 by $10\frac{1}{2}$ inches.
6. Hard drawing pencil, 5H or 6H.
7. Eraser with beveled edge.
8. A pad of emery paper for sharpening pencils.
9. A set of dissecting instruments which should include as a minimum the following articles:

   2 pairs of fine forceps, milled tips, 5 inch.
   1 pair of fine scissors.
   1 scalpel.
   2 teasing needles.
   1 millimeter rule.
   1 leatheret instrument case is desirable.

All other equipment and materials will be supplied by the laboratory.

LABORATORY REGULATIONS

Laboratory work begins promptly at the hour designated. It is essential that each person should recognize responsibility for the equipment furnished by the laboratory and the neatness of the place occupied. Each article of the equipment bears a number corresponding to the table assigned. After use it should be returned in good condition to its proper place. At the begin-

ning of the laboratory period report should be made to the instructor concerning any part of the laboratory equipment which is missing or out of repair.

Whether the laboratory is a clean, quiet, and comfortable place to work depends upon each member of the class.

All laboratory drawings and notes must be handed in at the close of each laboratory period. They will be corrected and returned at the next session. No credit will be given for such reports after they have been taken from the laboratory. Laboratory reports are judged with regard to *completeness*, *correctness*, and *neatness*. Technique and general attitude toward work and laboratory associates reveal the character of the worker.

In the upper right-hand corner each sheet of drawings or notes must bear the name of the student, the section, and laboratory number. Each sheet should also bear at the top and center the name of the study upon which it is a report.

RECORD OF OBSERVATIONS

Drawings.—Some record of studies made in the laboratory is always necessary either as a report upon the work accomplished or as information to be used in later study. When time permits there is nothing quite so satisfactory as an original drawing, for, as an eminent biologist long ago expressed it, "the best eye for a naturalist is the point of a lead pencil." But one need not be an artist in order to produce satisfactory outline drawings delineating laboratory observations. Indeed, most students who find their way into biological courses have had no previous training in drawing. The first attempt at drawing may be poor but it is likely that the fault lies in *poor observation* and *thinking* rather than in a lack of ability to produce simple diagrams. Improvement in observation is accompanied by a corresponding improvement in drawing. No attempt at drawing should be made until the object of study is understood. An attempt to draw will lead to closer observation.

Beginners often err in making drawings too small and attempting to represent too much detail. In the outlines which follow appropriate dimensions are suggested in connection with the drawings required. Each drawing should be so located upon the page and in its relation to other drawings which may be planned for the same sheet that a balanced appearance of the plate may be the result. Adequate space for all labels should be provided.

Perhaps the most common mistake made by beginners is a failure to give sufficient time to the study of proportions. No amount of care in representing details will compensate a poorly proportioned outline. The first outline sketch should therefore be made lightly to admit of erasures and additions as the study progresses. The appearance of drawings improves rapidly as one's sense of proportions becomes more dependable. The instructor may be asked to pass upon general proportions before details are represented. When the proportions of the outline seem satisfactory retrace the corrected outline with an unbroken clear line. Details of structure may now be located with constant comparisons of the drawing and the object. Use light lines at first, following with heavier ones later.

Keep in mind the fact that drawing is only a means to an end, *not the object* of laboratory work. Drawings better than words reveal to the instructor the student's progress and the difficulties he encounters.

The appearance of a good drawing may be spoiled by labeling in a careless manner. Good results may be obtained by considering the following suggestions:

1. Printed or written names of parts should be connected by guide lines with the parts they represent.
2. A broken line is preferable as a guide line.
3. Never allow guide lines to cross. As far as possible guide lines should be parallel. There is no substitute for a ruler in drawing these.

ABCDEFGHIJKLM

NOPQRSTUVWXYZ

abcdefghijklm

nopqrstuvwxyz

1234567890

4. Use as short a guide line as possible. Labeling on each side of a drawing is permissible, when necessary.

5. It is always desirable to print labels. As an aid for those who have not yet learned to print the accompanying sample of lettering is included.

Plates.—While the value of original drawings is recognized, it has become apparent that more than a minimum expenditure of time consumed in this way is utilized at the expense of a careful study of laboratory materials. With this in mind there has been prepared a series of plates with diagrams to be labeled or completed and labeled. What is lost in drawing is more than compensated by additional time for original study and for an individual demonstration-quiz. Before the close of every laboratory period each student should demonstrate to the instructor the results of his study and respond to questions. The plates when labeled may be used as a means of recording information for later use.

THE STUDY OF AN ANIMAL TYPE

In the study here outlined it is assumed that the objective is that of gaining a better understanding of the biological nature of the higher animals and man. At the outset of such a study it is necessary that one should acquire a clear conception of what is signified by the term "living individual" or "organism" as a center about which to organize zoological knowledge. An organism is something more than a mere spatial association of structural components, however orderly they may be arranged. What constitutes the body of the animal? How are the structural components of the body related? What are the services of the various parts and why are these services necessary? How are these diverse structures and their activities maintained and combined to form a unit and what is that unit? Studies along the lines suggested by such questions provide the basis for a comprehension of what is meant by the term "organism." These deliberations are, therefore, begun with the study of an animal type which will furnish the foundation upon which to place a conception of an organism. The frog is chosen as a convenient form, since it is a familiar animal exhibiting a moderately complex structure and in all fundamental respects of structure and physiology may be compared directly with man. The structural similarity is easily recognized by standing the frog upon its hind legs and making the comparison part for part.

In the frog and other complex animals it is possible to distinguish general regions of the body and directions within the body without reference to the movements of the animal or to any part of the environment. When such a state prevails the method of naming parts and directions is known as *intrinsic toponomy*. The following is a list of terms which will be employed according to this method of naming parts:

Dorsum, or back, that side of a crawling or quadrupedal animal normally away from the substratum (support) upon which it rests.

Dorsad, toward the back or in that direction.

Dorsal, pertaining to the back.

Venter, or belly, that side of the animal normally toward the substratum.

Ventrad, toward the venter or in a ventral direction.

Ventral, pertaining to the venter.
Cephalon, head, or that end which is foremost in locomotion.
Cephalic, pertaining to the head or foremost end.
Cephalad, toward the head or foremost end.
Caudal, pertaining to the tail or hindermost end.
Caudad, toward the tail or hindermost end.
Meson, the imaginary plane in the exact middle of the body dividing it into a right and left half.
Mesal, pertaining to the middle or mesal plane of the body.
Mesad, toward or in the direction of the meson.
Lateral, on either side of the meson.
Laterad, extending from the meson toward either side.
Ectal, pertaining to the surface of the body.
Ectad, from the inner mass of the body toward or in the direction of the surface.
Ental, pertaining to the interior of the body.
Entad, toward or extending toward the inner mass.
Proximad, toward the proximal or fixed end of an organ (*e.g.*, the shoulder is at the proximal end of the arm).
Distal, pertaining to the free end of an organ; (*e.g.*, the hand is at the distal end of the arm).
Distad, toward the free end.

Memorizing these terms as a set task is not advised. It is better to refer to the table when necessary to know the meaning of a term, thus learning it and its application at the same time.

It is to be emphasized that the frog is studied as a representative animal to illustrate the fundamentals of organization upon which our conception of an organism is based and the general principles of biology in their application to animal study. It is also studied to reveal the nature and architectural plan of a higher animal.

The study of the frog is enhanced by observations upon living specimens, several of which should be available in the laboratory.

Animals are noticeably different from plants in respect to the necessity of voluntarily approaching the food which they appropriate from the world about them.

Animals are rarely passively supplied with food. It is necessary for them to forage, hence the need of organs of locomotion. It is also necessary that the animal be in possession of some special means of gathering information concerning objects and forces about it in order that it may harmoniously adjust itself to these environmental conditions. The manner in which these and other similar problems have been solved in the animal body may be taken as an expression of the object in studying the exterior of the frog.

The body comprises two regions, *head* and *trunk*. The arms and legs are regarded as *appendages* of the latter. Note the color pattern, the distribution and intensity of color, and any striking markings upon the body.

When the frog once confronts its food it is still in need of provisions for getting the food into its digestive organs. Reflect upon the usefulness of hands and jaws in this service, bearing in mind the non-grasping nature of the hands.

In the forelimb or arm, beginning at the body, identify upper arm or *brachium;* the shoulder joint and its freedom of movement; forearm or *antebrachium;* elbow joint and its movements; *wrist;* hand or manus with *digits* (fingers). Why is your own hand efficient as a prehensile (grasping) organ? What is lacking in the frog's hand? What is suggested by the structure, form, attitude, and flexibility of the arm in its service to the frog?

In the hind limb identify *thigh*, *shank*, *ankle*, *foot* or *pes* with *digits* (toes). Locate and compare the hip, knee, and ankle joints with the corresponding joints of the arm. How many digits in the foot? Are the digits of the hand and foot similar in arrangement and in length according to numerical position? Compare the palm of the hand and sole of the foot; the heel with the corresponding part of the hand. Do these peculiarities of hands and feet suggest any sort of advantages to the animal?

If not already familiar with both the terrestrial and aquatic modes of locomotion of the frog make such observations upon living specimens. Attempt a correlation of these observations with the position of the limbs when at rest, the movements and service of parts in active locomotion, determining, if possible, a general locomotor function which may be assigned to each pair of limbs, taking into consideration the need of buffers, propellers, steering devices, leverage, rigidity, and flexibility. If the limbs meet such needs, explain how it is done.

Organs of Special Sense.—The awareness of the frog to surrounding conditions can be readily determined by observation and simple experiments performed upon the living animal. The means of gathering information from the surroundings are sense organs (receptors) either located within restricted areas as the organs of vision, smell, taste, and hearing or scattered as the organs of touch and other senses. Some of these organs cannot be observed by macroscopic study. Touch corpuscles and fine *nerve endings* in the skin render the frog sensitive to

contact, changes in temperature and moisture, the chemical nature of surroundings, and even light.

A substance which stimulates the sense of taste must be in solution. It penetrates the surface membrane of the mouth and stimulates special taste organs. In the frog these organs occur apparently on the floor and roof of the mouth and particularly on the flattened surfaces of special mushroom-shaped elevations, *fungiform papillæ,* on the tongue. The organs of taste, like the organs of touch, cannot be made out in a macroscopic study but they belong to the surface of the body, since the mouth lining and the skin form a continuous sheet of material identical in origin.

Locate the two nostrils or *external nares* on the upper surface of the snout. Pass a beaded bristle through one and observe that it comes out of a round opening in the roof of the mouth cavity. This is the *internal nostril* or *naris.* The canal between the external and internal nares serves for the passage of air on its way to and from the mouth cavity. This short canal is enlarged during its course to form the *olfactory sac* the lining of which is thickened and otherwise modified to form the receptive organ of smell, *olfactory sense organ.* Gases given off from odorous bodies are detected by these organs. The olfactory sense organs constitute the doorway through which the animal communicates with the outside world as regards the nature of gases (odors) which surround it. The advantage of an organ of smell located in the walls of the breathing passages is obvious.

Posterior to the external nares are the large prominent *eyes,* doorways for communication by means of light waves. In each may be distinguished the *iris,* surrounding a central opening, the *pupil.* By the proper manipulation with forceps eyelids may be discovered. Determine their locations and relative size and importance in covering and protecting the eye. The *nictitating membrane* is a thin, transparent "third eyelid" which is really an outgrowth of the lower lid. This may be observed in a living animal. In most animals, the nictitating membrane when present is distinct from the true lower lid. It reaches a high degree of development in birds, where it may be stretched over the whole front of the eye. In man it is represented by a vestigial fold at the inner angle of the eye. What advantage may reside in the presence and transparency of the nictitating membrane? Compare the position of the eyes in frog and man.

Can both eyes focus together upon the same object (binocular vision) or each eye separately (monocular vision)? Why? Ask the instructor for a method of determining your own angle of vision. Represent by a simple diagram. Looking down upon the top of the frog's head determine the angle of vision of each eye. Represent by a diagram. What do you judge to be the relative merits of the two kinds of vision?

On the side of the head caudad of the eye is a flat disk, the *tympanum* or eardrum, which responds to vibrations impinging upon it. By careful manipulation determine the nature and attachments of this membrane. Near the center on the inner surface of this membrane there is attached a small rod, the *columella,* which transmits the vibrations of the tympanic membrane to the *inner ear* or receptive organ of the sense of hearing. Carefully remove the cephalic third of the membrane as an aid in discovering its inner relations. If difficulties are encountered ask the instructor for assistance.

In the mesal line of the head, slightly cephalad of the level of the eyes, there is a small light-colored spot termed the *brown spot.* Its significance will be mentioned later. For the present locate it only.

The *anus* (the caudal opening of the intestine) appears to be located on the dorsal side rather than at the exact caudal end or on the ventral side.

A survey of the sense organs (receptors) reveals them as organs located at the surface or in direct communication with the surface. Bearing in mind the general service of the sense organs what is the fitness of their location?

Letting a horizontal line represent the frog's body, locate, by means of numbers or symbols, the organs of the five special senses (vision, smell, hearing, taste, and touch). What is the result? Of what obvious advantage to the animal?

Identify the *dermal plicæ* or thickened folds of skin extending caudad from the posterior angle of the eyes. These folds lodge important skin glands.

Make a drawing of the dorsal aspect of the frog, life size (if the frog be of medium size), **representing the features mentioned in this study.**

THE FROG'S SKELETON

For this study prepared skeletons are provided. They are fragile and therefore must be handled with care. The value of each is such that a charge must be made for any breakage which is the fault of the user.

The need of a firm and, at the same time, flexible support for the body is apparent in considering such functions as locomotion in which rigid support and precision of movement are paramount. Such functions, as well as many others, reside in the skeletal framework of the body. The skeleton as a whole comprises two main divisions: (*a*) the axial skeleton and (*b*) the appendicular skeleton.

Axial Skeleton.—Extending along the middle line or main axis of the body there are found the *skull* and *vertebral column* constituting the *axial skeleton*.

The Skull.—Recalling the shape of the frog's head examine the skull and determine the various aspects (*i.e.*, dorsal, ventral, cephalic, caudal, lateral). The most conspicuous feature of the skull is the presence of a relatively enormous and somewhat rectangular space on either side of the middle port on. These spaces mark the location of the eyes and are known as the *orbits*. Through two smaller spaces immediately cephalad of the orbits the nostrils open.

The skull placed at the cephalic end of the axial skeleton comprises (*a*) the *cranium* which encloses the small brain and occupies the mesal region between the orbits. Associated with the cranium are combinations of bones and cartilages forming protecting encasements for certain sense organs. These are called "sense capsules." They occur in pairs: the *olfactory capsules* surrounding the olfactory organs at the cephalic end of the cranium and the *auditory capsules* on either side at the caudal end. (*b*) the *visceral skeleton* includes the framework of the jaws and hyoid apparatus (tongue support). In the caudal end of the cranium is an opening, the *foramen magnum*, through which the spinal cord extends from the brain. Note the lines (sutures) formed by the meeting of bones. Determine the location of teeth. Identify the slender bone, *columella,* connecting the

ental surface of the tympanum and inner ear. This is the bone already seen when studying the tympanum. It transmits vibrations from the tympanum to the auditory sense organ encased within the auditory capsule.

The margin of the upper jaw is formed in front by the *premaxillaries*, one on either side, each of which sends a process in a dorsal direction, and on the side by the *maxillary*. Both premaxillary and maxillary bones bear teeth.

The lower jaw is formed chiefly by the long *dentaries*, one on each side.

Vertebral Column.—The vertebral column is composed of a series of irregular bones including nine elements called *vertebræ* and a caudal elongate bone, the *urostyle*. The first vertebra, or *atlas*, is slightly modified because of its articulation with the skull. Determine its peculiarities in this respect. The last vertebra possesses a lateral projection (transverse process) which is somewhat enlarged and serves as the attachment for the skeleton of the hind limb. This vertebra is termed the *sacral vertebra*.

Select the fourth vertebra as a typical one for study and identify the following parts:

a. Centrum, the solid cylindrical portion of the vertebra. This part is always ventral to the other parts.

b. Neural canal, the enclosed space or cavity dorsad of the centrum and lodging the spinal cord.

c. Neural arch, the bony portion forming the sides and roof of the neural canal.

d. Neural spine, the dorsal projection from the middle of the neural arch.

e. Transverse processes, lateral projections from the junction of neural arch and centrum.

f. Zygapophyses, cephalic and caudal projections from the base of the neural arch. They serve to yoke the vertebræ together.

The Appendicular Skeleton.—This division of the skeleton comprises (*a*) the bones of the free limbs, or those portions which may be seen projecting beyond the general surface of the body, and (*b*) the girdles which are embedded within the body walls and with which the skeleton of the free limb is articulated.

The pectoral girdle (shoulder girdle) completely encircles the body at the level of the arms. Each half consists of two portions, dorsal and ventral, which meet at the socket of articulation of

the arm bones. This socket or articulating excavation in the girdle is known as the *glenoid fossa.* In the middle line of the ventral portion the caudally projecting bony element is the *sternum.*

The skeleton of the arm consists of the *humerus* in the upper arm. In the forearm there are two bones side by side, the *radius* on the thumb side and the *ulna* on the other side, as in man. In the frog the radius and ulna are fused into one solid bone, the *radio-ulna.* Distad of (beyond) the lower arm is the *wrist* or *carpus,* containing six small bones known collectively as *carpals,* arranged in two rows. The proximal row, situated at the end of the radio-ulna, includes two elements; the distal row, three. The sixth element is located on the inner side of the wrist. The hand comprises five *metacarpal bones,* which support the palm and at their ends articulate with the fingers. The first digit (thumb) is absent. Its metacarpal is small.

The humerus is a good example of a long bone serving as a lever. The enlarged ends formed of cartilages are known as the *epiphyses,* the *shaft* being the narrower portion between. The proximal end of the humerus rests in the glenoid cavity, forming a ball-and-socket joint, and is held in position by muscles and ligaments. The strong ridge on the proximal portion of the shaft is the *deltoid ridge* and serves for the attachment of muscles.

The distal end of the radio-ulna displays very obvious traces of its double origin. The proximal end is *hollowed* out for articulation with the humerus, and the ulnar part is produced proximally to form a projection, the *olecranan,* which fits over the rounded end of the humerus at the elbow.

The *pelvic girdle* is shaped like a V with the open part directed cephalad. On each side the arm of the V is a distinct bone, the *ilium.* The ilium of each side articulates with the transverse process of the ninth vertebra. The ilia fuse with one another at the caudal end where there is formed a circular platelike portion excavated at its center. The cephalic half of this plate is formed by the fused ilia. The caudal margin is formed by the *ischia* (singular *ischium*) one on each side, which have joined at the middle line. The ventral margin of the plate is occupied by the *pubes* (singular, *pubis*) which like the ischia have joined at the middle line to form a triangular mass of cartilage. In old frogs this element becomes calcified. The ilium, ischium, and pubis all take part in the formation of the *acetabulum,* the excavation in which the leg skeleton articulates with the girdle.

The skeleton of the leg consists of (1) a thigh bone or *femur*, (2) a lower leg bone, the *tibio-fibula*, formed by the united *tibia* (great toe side) and *fibula* (outer side), (3) an ankle region including the *tarsal* bones, two long proximal elements, and three imperfectly ossified elements forming a distal row, (4) five *metatarsal* bones, and (5) five *toes*, or *digits* the segments of which are called *phalanges*. Note the rudimentary sixth digit, the *prehallux*, on the inner side of the first toe.

After demonstrating to the instructor all the parts of the skeleton mentioned in the preceding paragraphs, label the same parts on the proper plates.

SYNOPSIS OF SKELETAL DIVISIONS

- Skeleton
  - Axial
    - Skull
      - Cranium
        - Brain case
        - Olfactory capsules
        - Auditory capsules
      - Visceral skeleton
        - Upper jaw
        - Lower jaw
        - Hyoid and larynx
    - Vertebral column (including ribs when present)
  - Appendicular
    - Girdle
      - Shoulder
        - Scapula and suprascapula and a number of elements not elsewhere mentioned in these outlines; *viz.*, coracoid, procoracoid, epicoracoid, clavicle, episternum, omosternum, sternum, xiphisternum
      - Hip
        - Ilium
        - Ischium
        - Pubis
    - Free limb
      - Arm
        - Humerus
        - Radio-ulna
        - Carpals
        - Metacarpals
        - Phalanges
      - Leg
        - Femur
        - Tibio-fibula
        - Tarsals
        - Metatarsals
        - Phalanges

THE MUSCULAR SYSTEM

All movements of an animal are the results of muscular activity. The *skeletal* muscles constituting a great portion of the body mass are, in most cases, attached to some part of the skeleton. These are the muscles to which reference is made when the term "muscular system" is used. The skeletal muscles, because of a peculiar banded appearance of their component fibers, are also termed *striated muscles.* There is another type of muscle associated with internal organs such as the walls of blood vessels and the intestine. Since these muscles are so arranged that they extend in a circular fashion about cavities they are known as *hollow muscles.* Their fibers are not crossed by bandlike striations, and thus they present a smooth appearance. They are therefore termed *non-striated* or *smooth muscles.* The skeletal muscles for the most part can be controlled by the organism and are therefore often termed *voluntary* muscles in contrast to the hollow or involuntary muscles which are not so controlled.

The middle portion of a muscle which shortens and increases in diameter during contraction is called the *belly.* From this part *tendons* extend to the bones for attachment. The two ends of the muscles are attached to different bones. Otherwise the contraction would come to naught as far as moving parts of the body are concerned. The bones serve as levers and fulcra, the muscles as the power. A thin, transparent, glistening membrane, *fascia,* adheres closely to the muscle. The less movable or fixed attachment of a muscle is called its *origin;* the movable end, the *insertion.*

The primary reason for the study of a muscle is that of determining its action, its name and attachments serving as a means to this end. After the attachments are known it is not ordinarily a difficult matter to determine the action.

Muscles exhibit force or perform work during contraction only. When a body part has been moved in one direction by a given muscle or group of muscles acting in unison there will be found another muscle or group of muscles for the movement of the body part in the opposite direction. This is called the *antagonistic*

action of muscles and such a muscle or muscle group is known as the *antagonist* of its opponent. The application of this principle facilitates the study of muscles to a considerable degree. The contraction of a muscle is in the direction of the long axes of its fibers which serves as an index to the particular work which a given muscle or group of muscles may perform in producing movement in any part of the body.

Remove the skin from both the body and its appendages. In doing so it will be noted that over the greater part of the body the skin is not attached to underlying parts but that along certain narrow lines there are firm attachments which must be cut in order to free the skin. This forms underneath the skin great spaces circumscribed by the lines of attachment. These spaces in the living frog are filled with lymph and from their location and contents are termed *subdermal lymph spaces*.

The removal of the skin exposes the superficial muscles of the body. The muscle masses may be improved for study by rinsing in a current of water and at the same time brushing the surface with a wad of absorbent cotton. During the progress of the study keep the muscles moist.

A study of the muscles is not difficult but requires careful observation and thought. It is necessary to *see* what you look at and *think about* what you see.

Manipulate the body of a live frog or one recently killed and determine those places where there is the greatest amount of movement or flexibility. A list of such places will be found convenient for later use. Observing the dorsal surface of the frog one notes that in certain places the muscles are massed while in others they are almost absent. Considering the dorsum of the body and the arm and leg of one side only, excluding hand and foot, there are six of these masses. Identify and give each a name which will indicate its location and so label the proper figure of the plate representing the superficial (surface) muscles. Determine and express the relation of these muscle masses to points of flexibility.

Repeat these observations upon the ventral side. Express results.

Study these muscle masses in the light of what has been said above concerning muscle antagonism. Results?

The Study of an Individual Muscle.—For studying the fibers and attachments of individual muscles the dissecting microscope may be found useful. The *gastrocnemius* muscle which forms

the calf of the leg is selected for study. Its name means *stomach* plus *leg*, which is a reference to the enlarged belly of this muscle. Lay out the frog with its dorsal side upward. Pull the right foot caudad so as to straighten the leg. The gastrocnemius will be found to occupy the whole inner border of the shank between knee and heel. With an orange stick or blunt instrument separate it from the adjacent muscles. The origin of the gastrocnemius is by two tendinous heads, one, a short clearly visible tendon extending to a broad sheetlike tendon spreading over the distal end of the femur and knee joint. This is the equivalent mechanically to an attachment upon the end of the femur itself. The other head is attached by a short tendon exposed by rotating the shank slightly and exposing the "hollow" of the knee. Here the tendon of this head joins other tendons which are attached to the end of the femur. Thus the two heads of the gastrocnemius are seen to have their origin virtually upon the femur. This muscle tapers toward the heel where a strong tendon extends over the ankle joint to become inserted upon the sole of the foot.

In the origin, extent, and insertion of the gastrocnemius muscle one sees provision made for its action which is that of flexing the leg at the knee and extending the foot. In addition to its double function one sees that it extends over two joints (knee and ankle) instead of one. Muscles which extend over two joints and possess a double function act in conjunction with other muscles, all of which increases their mechanical advantage. This is to be correlated with providing the power for leaping.

Label this muscle and its parts upon the plate showing the muscles of the dorsum. Upon the plate point out the reasons for regarding the gastrocnemius as a two-joint muscle with a complex action. Label all parts to which reference is made.

The action of a muscle is the movement or movements in the body resulting from its contraction; that is, what the muscle *does* is spoken of as its *action*. When a part of the body bends, as, for example, the leg bends at the knee, it is said to be *flexed* and the muscle or muscles concerned with the flexing are called *flexors*. When a part of the body is straightened it is said to be *extended* and the muscle or muscles participating in such movements are called *extensors*. With the proper attachments a given muscle may serve as a flexor for one part and at the same time as an extensor for another. Muscles may, there-

fore, be classified according to their actions as in the following categories:

Flexors, bend a part.
Extensors, straighten a part.
Adductors, move one part toward another or toward the mesal plane of the body.
Abductors, move one part away from another or away from the mesal plane.
Rotators, rotate a part upon its axis.
Depressors, lower or depress a part.
Levators, elevate or lift a part.

The action of individual muscles or muscle masses can be demonstrated by stimulation with a galvanic current upon a fresh specimen. The experiments will be performed by the instructor, the class making the observations and formulating explanations and answers to questions.

By either experiment or judgment of the action of muscles formed through observing the direction of fibers and attachments select individual muscles exemplifying each category mentioned above and label accordingly the proper muscles on the plate.

If time permits, other muscles may be studied under the direction of the instructor.

THE ORAL CAVITY

An etherized frog is best adapted to this study. Note that the jaws fit together very tightly. Open the mouth to its full extent and note the wide mouth (oral) cavity the posterior portion of which, the *pharynx*, is continued caudad into the *esophagus*. In the roof of the mouth identify the structures mentioned in the paragraphs which follow.

There are two groups of teeth found in this animal. The *maxillary* teeth form a row about the edge of the upper jaw where they are borne by the maxillary and premaxillary bones. The *vomerine* teeth form two small patches in the cephalic part of the roof of the mouth and near the middle line. They are attached to the vomers (vomerine bones).

Locate again the *posterior nares* lying laterad of the two groups of vomerine teeth. The *eustachian tubes* open into the oral cavity through large apertures at the sides of the posterior part of the mouth cavity. Each tube enters a slightly dilated chamber, the tympanic cavity (or middle ear), which lodges the columella and is closed externally by the tympanic membrane already seen in the study of the surface of the head. Pass a beaded bristle through the opening of one of the eustachian tubes and determine the results. If necessary make a slit in the tympanum.

Two rounded prominences on the sides of the roof of the mouth are caused by the eyeballs. Press down upon one of the eyes with the finger and note results.

The floor of the mouth cavity presents several features to be noted. The lower jaw, which lacks teeth, forms a bony margin for the floor of the mouth. The greater extent of the floor is soft and supported by a cartilaginous plate, the body of the *hyoid*. The *tongue* is attached to the cephalic portion of the floor and has its free bilobed end turned caudad toward the *esophagus*. If this organ is drawn cephalad the structures about to be described may be seen more readily.

The *glottis*, or aperture leading into the lungs, is a longitudinal slit upon the apex of a prominence, just cephalad of the esophagus. It is supported laterally by the arytenoid cartilages

which in the frog represents the voice box of higher animals. These cartilages cannot be seen without dissection. Just beneath the corners of the mouth are the openings of the *vocal sacs* in the male.

Upon the plate showing the roof and floor of the mouth in outline represent and label all the oral structures mentioned above.

THE VISCERA

Lay the frog on its back under water. By passing pins through the limbs fasten it to the wax bottom of a tray. Cut through the skin, along the middle line, the whole length of the ventral surface. Separate the skin from the underlying parts The muscles of the ventral body wall and the pectoral girdle should now be exposed. Note a dark blue streak running along the mesal longitudinal line just beneath the muscular walls. This line represents the location of the *ventral abdominal* vein. With forceps pinch up the muscular body wall and with scissors cut through it into the *celom* (body cavity) a *little to the left of the median line.* Continue this cut caudad to the end of the body and cephalad to the jaws, cutting through the pectoral girdle with strong scissors, taking care not to injure the parts beneath. If incisions taking a lateral course be made through the body wall and skin in the region of the pelvic girdle the two resulting flaps of body wall may be lifted and pinned so as to expose the abdominal viscera (organs). It may also be necessary to clip off the mesal portions of the two halves of the pectoral girdle in order more clearly to reveal the relations of organs in the cephalic part of the body cavity. In this study locate and identify all structures mentioned, whether directed to do so or not.

Note that the body cavity is lined by a smooth glistening membrane, the *peritoneum.* The various organs are suspended from the dorsal walls by membranes called *mesenteries.* These are double folds of peritoneum.

The Heart.—With great care free the pectoral girdle from the underlying muscles and blood vessels. Note the *pericardial cavity* and examine the heart with a view to the identification of the following parts:

1. The thin-walled *auricles* form the cephalic and dorsal divisions of the heart. They are named according to their location upon the right or left side. Blood returning from the several

systems of the body is received by the right auricle and passed on to the ventricle. The left auricle receives blood coming from the lungs and passes it on to the ventricle.

2. The thick-walled *ventricle* is caudad of the auricles; it is conical in form, with the apex pointing caudad.

3. The *truncus arteriosus* is a tube arising from the right cephalic border of the ventricle and extending obliquely cephalad across the auricles. This trunk is the only course followed by the blood upon leaving the heart for the several regions of the body.

4. Lift up the ventricle and turn its apex cephalad. Do this very gently and do not allow too much strain to come upon the parts disturbed. Note three large vessels (veins) converging in their course toward the dorsal side of the heart. The thin-walled compartment which all three veins enter is the *sinus venosus* or receiving station for the blood coursing back to the heart from the body where it has performed its work. The sinus venosus communicates with the right auricle which is the final division receiving blood from the various systems of organs.

On the plate showing the diagram of the heart label the parts.

The Abdominal Viscera.—The *liver* is a large reddish-brown organ behind and at the sides of the heart. It is either trilobed or bilobed with the much larger left lobe further subdivided into two parts. The *lungs* are two thin-walled but elastic sacs at the sides of the heart; they lie dorsad of the liver and are often concealed by it when viewed from the ventral side. The glottis, noted in the study of the floor of the mouth, is the opening from the mouth cavity into the larynx communicating with the lungs.

Study the location and relations of the reproductive organs. If your specimen is a female the ovaries will be seen as two large bodies of irregular shape, each comprising a mass of spherical black and white eggs, appearing like small shot. When fully developed the eggs break through the walls of the ovaries into the body cavity and find their way in a cephalic direction to the openings of the paired oviducts. These are two long very much convoluted tubes with thick white walls, lying at the sides of the body cavity. After the relations of ovaries and oviducts have been noted those of the left side may be removed.

Turn the liver forward and note the *stomach* concealed by the left lobe. Pass the handle of a needle through the mouth and down the esophagus into the stomach which is an enlarged portion of the alimentary canal between the esophagus and

intestine. The stomach is narrower at its caudal end and is demarcated from the intestine by the distinct circular *pylorus* or *pyloric valve.* Beyond the pylorus is the small intestine, consisting of the *duodenum*, that part of the intestine lying parallel to the stomach, and the *ileum,* which is the slender convoluted portion of the small intestine continuous at its distal end with the *large intestine.* This passes without noticeable change of size into the *cloaca* which is the most caudal division of the alimentary canal. Locate the *urinary bladder* which is a thin-walled, bilobed sac opening through the ventral walls of the cloaca.

The *gall bladder* is a small spherical green sac lying between the right and left lobes of the liver. The *pancreas* is a pinkish irregular mass lying in the loop between the stomach and the duodenum and best seen by turning the whole loop toward the head. The pancreatic ducts are numerous but too small to be observed readily. They open into the slender *bile duct* which passes through the pancreas on its way from the liver and gall bladder to the duodenum. Bile secreted by the liver passes down the hepatic ducts into the common bile duct and is stored in the gall bladder until it is discharged into the intestine. Bring gentle pressure to bear upon the gall bladder. This will force bile into the *common bile duct* which may then be located. It is about the size of a large thread and enters the duodenum at a point about one-half inch beyond the pylorus and on the inner or concave side of the loop formed by the stomach and duodenum.

The *spleen* is a small, dark-red, ovoid body lying in the mesentery, opposite the beginning of the large intestine. It is not a part of the digestive system.

After the above-mentioned organs of the digestive system have been located, turn the alimentary canal and its appendages over to the left side of the body cavity so that the structures of the excretory and reproductive systems may be studied on the right side.

The *kidneys* are a pair of elongate, reddish-brown organs attached to the dorsal body wall, close to the middle line, one on each side of the vertebral column. They lie in the large lymph space dorsad of (outside) the peritoneum The *ureter,* or duct of each kidney, is a light-colored tube appearing to arise from the outer caudal margin. It extends caudad to open through the dorsal wall of the cloaca. On the ventral surface and partly

buried in the substance of the kidney is a narrow yellowish band, the *adrenal gland*, where there is produced a secretion of importance to the life of the organism. Usually closely associated with the anterior ends of the kidneys is a pair of bright yellow finger-like tufts of flattened processes, the *fat bodies.* These vary much in size at different seasons of the year.

The reproductive system consists of the sex organs, or *gonads*, in which the sex elements are lodged, and ducts extending to the cloaca.

In the male frog the gonads are termed the *testes* (singular, *testis*). They may be seen as small, yellow, bean-shaped organs located at the ventral surface of the kidneys. A number of slender ducts, the *vasa efferentia*, may be made out in the mesentery between each testis and the inner border of the corresponding kidney. Spermatozoa (the male reproductive elements) pass through these ducts into the tubules of the kidneys and thence to the cloaca by way of the ureters. A tube conducting the spermatozoa from the testis toward the exterior is called a *vas deferens.* It will be seen that in the frog the ureter serves both as ureter and vas deferens. In many species, and especially is this true of the leopard frog (*Rana pipiens*), the male possesses a vestigial oviduct which appears as a wavy white tube on each side, laterad of the ureters.

It will be noted by those who have female frogs that the ovaries lie in the same relative position as do the testes of the male but vary considerably in size and shape according to the season of the year. The most conspicuous organs in the celom of a mature female in the spring are the ovaries, bearing great numbers of ova. The ovaries bear no such relation to the kidneys as do the testes in the male but, as already mentioned, shed their eggs into the body cavity whence they make their way to the openings of the oviducts, which they follow to the exterior. The funnel-shaped mouth of an oviduct may be visible close to the outer side of the root of the lung of the same side. The opening of the oviduct through the dorsal wall of the cloaca should be identified. Unlike the male, the female possesses genital ducts independent of those of the urinary organs.

After locating and studying all organs and structures described above, label those shown on the plate and add any which may not be represented. The reproductive system on the right side only need be shown.

THE BLOOD SYSTEM

The circulatory system is a closed series of vessels filled with blood and ramifying through all parts of the body. Its main parts are (1) the *heart*, which by its muscular activity propels the blood through the vessels, (2) the *arteries*, which are the vessels conducting the blood from the heart to all parts of the body, (3) the *veins*, which return the blood from the various parts back to the heart, and (4) the *capillaries*, a system of very small vessels forming the transition from arteries to veins. It is during the course of the blood through capillaries that it performs its main service in the body.

The order in which blood vessels are studied will depend upon the way in which the study specimens have been prepared. If one specimen is to serve for this whole study the veins, whether injected or not, should be studied before the arteries. The following directions are based upon the use of two specimens. For the study of the arteries the vessels are filled with a yellow starch mass injected through the ventricle which abnormally distends this compartment of the heart.

Place the frog on its back in a wax-bottomed tray and extend the slit, made in injecting the animal, cephalad to the tip of the lower jaw and caudad to the pelvic girdle, cutting through both the skin and body wall. Note the *ventral abdominal vein*, which may be seen as a streak along the midventral line. Carefully separate this vessel from the body wall by gently tearing its attachments. Next, make transverse slits through the skin and body wall to the right and left of the midventral incision about a quarter of an inch cephalad of the caudal end of the body cavity. Turn back the flaps, thus formed. At this point students dissecting a female frog should remove the ovary and oviduct of the animal's left side. Care must be used in separating the blood vessels from the body wall, since they will break if placed under too much strain. Use *judgment* with regard to the mechanics of dissecting. If more convenient for the worker the frog may be removed from the liquid and held in the hand.

The Arteries.—While the numerous blood vessels of the body may be confusing at first, there is no need for viewing their study as an impossible or even a difficult task. There are certain observations which, if kept in mind, will facilitate this study:

1. The heart is the central organ of the whole blood system.
2. Arteries convey currents away from the heart.
3. Veins convey currents toward the heart.
4. The organs of every region of the body must be supplied with blood from the heart.
5. The organs of every region must be drained of blood toward the heart.

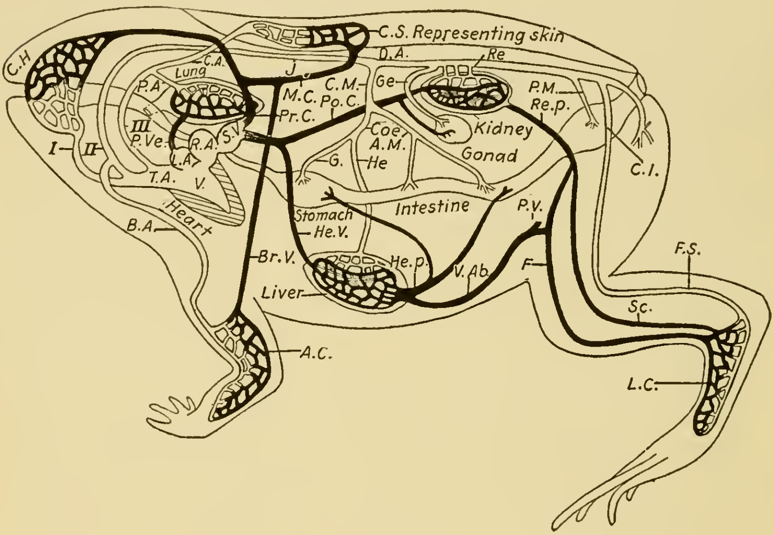

FIG. 1.

Based upon the distribution of arteries to the various organs the body may be divided into three main regions: (1) the head, (2) the lungs and skin, (3) the trunk, arms, and legs. Each of these regions is supplied on each side by its own main trunk leaving the heart. Figure 1 is a schematic representation of the three regions and the arteries which supply them and as well the main vessels of the whole body. It should be kept in mind that this figure is *schematic* and not intended to show actual details. By a careful study of this schema aptitude will be acquired for the dissection and study of the vessels themselves.

Arising from the ventricle of the heart and passing obliquely across the auricles is the large vessel, *truncus arteriosus*, referred to above. Note the divisions of the truncus arteriosus into right

FIG. 1—DIAGRAM OF THE BLOOD VESSELS OF THE FROG.

This diagram is schematic and not intended to show details.

The *capillary networks* of various regions are represented as follows: A. C., arm; C. H. head; C. S., skin, L. C., leg.

ARTERIES (unshaded)

Main Vessels:—

I. Carotid arch supplying the head region.
II. Aortic arch supplying the arm-trunk-leg region.
III. Pulmo-cutaneous arch supplying the lung-skin region.

Subordinate Vessels:—

A. M., anterior mesenteric artery
B. A., subclavian artery
C. A., cutaneous artery
C. I., common iliac artery
Coe., coeliac artery
C. M., coeliaco-mesenteric artery
D. A., dorsal aorta
F. S., femoral and sciatic arteries
T. A., truncus arteriosus
G., gastric artery
Ge., genital artery
He., hepatic artery
J., junction of aortic arches of either side to form the dorsal aorta
P. A., pulmonary artery
P. M., posterior mesenteric artery
Re., renal arteries

VEINS (solid black)

Main Vessels:—

Po. C., post cava vein draining the trunk-leg region.
Pr. C., precava vein draining the head-arm-skin region.
P. Ve., pulmonary vein draining the lung.
S. V., sinus venosus receiving blood from whole body except the lungs.

Subordinate Vessels:—

Br. V, brachial vein
F., femoral vein
He. V., hepatic vein
HeP., hepatic portal vein
M C., musculo-cutaneous vein
P. V., pelvic vein
Re.p., renal portal vein
Sc., Sciatic vein
V. Ab., Ventral abdominal vein

HEART

L. A., left auricle; R. A.; right auricle; V., ventricle

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cutaneous artery may then be seen. (*b*) The *pulmonary artery*, following a somewhat wavy course along the outer side of the

The Arteries.—While the numerous blood vessels of the body may be confusing at first, there is no need for viewing their study as an impossible or even a difficult task. There are certain

By a careful study of this schema aptitude will be acquired for the dissection and study of the vessels themselves.

Arising from the ventricle of the heart and passing obliquely across the auricles is the large vessel, *truncus arteriosus*, referred to above. Note the divisions of the truncus arteriosus into right and left branches, each of which again divides into three arches. Each of these arches supplies one of the regions mentioned above. In order to gain a clear view of these three arches and the various arteries arising from them, it will be necessary very carefully to remove the veins and other structures which overlie them. *Never take hold of a blood vessel itself with forceps. Handle it by seizing the supporting or associated tissues.* Begin at the truncus arteriosus and trace out the arches and arteries of the left side of the animal only, since the two sides are alike, reserving the right side for the study of the veins.

The branches of the main arches and their subdivisions are as follows:

1. The *carotid arch* (Fig. 1, I) is the most anterior of the three. It extends dorsad around the side of the esophagus and soon divides into two branches. A small, vascular swelling, the *carotid gland*, surrounds the base of these branches and often prevents the injecting mass from filling the vessels. The two branches are:

a. The *external carotid artery*, a small vessel supplying the tongue and floor of the mouth;

b. The *internal carotid artery* extending around the œsophagus to its dorsal side, then cephalad into the roof of the mouth along the base of the skull. Locate its origin from the carotid arch. Cut the lower jaw at the angle of the mouth. Peel the lining from the roof of the mouth and note its distribution in this region.

Which general region of the body is supplied by the carotid arch?

2. The *pulmo-cutaneous arch* (Fig. 1, III) is the third and most caudal of the three arches. It will be described and dissected at this point, since its branches are likely to be destroyed in the dissection of the middle arch. The pulmo-cutaneous arch divides at about the level of the carotid gland into (*a*) the *cutaneous artery* supplying the skin. Its course can be readily traced. Slit the skin along the middorsal line and turn it back until the shoulder region is visible. The course and distribution of the cutaneous artery may then be seen. (*b*) The *pulmonary artery*, following a somewhat wavy course along the outer side of the

whole length of the lung, gives off branches to its walls. When labeling this arch on any of the drawings state the region which it supplies.

3. The *aortic arch* (Fig. 1, II), the middle of the three main divisions, extends somewhat obliquely around the esophagus to its dorsal side and unites with its fellow of the opposite side at about the level of the cephalic end of the kidney to form a single vessel, the *dorsal aorta*. In order to expose the dorsal aorta pierce the peritoneum about the left kidney and gently pull the kidney away from the body wall so as to expose the large lymph space in which this vessel runs. The aortic arch and its branches in the region of the pectoral girdle can best be studied from the side.

Before the union of the two arches to form the dorsal aorta there are at least four branches given off, but attention is drawn to one only, the large *subclavian artery* which arises at about the level of the shoulder and extends laterad to supply the arm.

Branches given off after the union of the two arches to form the dorsal aorta are:

a. The *cœliaco-mesenteric artery*, a large median vessel arising near the union of the two arches, or sometimes from the left arch just before the union, and supplying the stomach and intestines. This vessel in its turn gives rise to two important branches: (1) the *common cœliac artery*, whose branches supply the stomach, liver, and gall bladder; and (2) the *mesenteric artery*, whose branches supply the intestine and spleen.

b. The *urogenital arteries*, four to six in number. These are small vessels which arise from the ventral surface of the dorsal aorta between the kidneys. They supply the kidneys, the reproductive organs and their ducts, and the fat bodies. There are other small arteries leaving the dorsal aorta, but the study of these may be omitted.

c. The *common iliac arteries* are large vessels formed by the division of the dorsal aorta near the level of the caudal end of the kidneys and are distributed to the legs. Cut the muscle mass on either side of and along the urostyle, which can then be elevated and removed along with the attached muscles. Trace the artery in its course down the leg.

A recently etherized frog is necessary for the study of the veins. If the veins are injected the venous system should be studied first.

The Veins.—Although the injected arteries of the frog studied are decidedly larger than uninjected veins, the latter in their normal state are larger than arteries. As a rule, the veins lie nearer the surface than do the arteries.

Keep in mind the fact that the ventricle is the compartment of the heart which forces blood out through the arterial system to all parts of the body. It receives blood from the auricles only. Four veins return blood to the heart; three open into the sinus venosus and thence into the right auricle; while one, the *pulmonary vein*, opens directly into the left auricle.

As in the case of arteries in their relations to general regions which they supply, one finds similar relations of the larger veins and regions which they drain back toward the heart. The vein regions differ slightly in the combination of parts from the artery regions. There are three, as follows: (1) the head, arms, and skin, (2) the lungs, (3) the trunk and legs. Two of these regions may be subdivided. The *head-arm-skin* region according to the relations of the main vessels draining them is divisible into (*a*) *head* and (*b*) *skin-arm* divisions. The trunk-leg region similarly comprises (*a*) *dorsal-trunk* and (*b*) *ventral-trunk* divisions.

Throughout the study of the veins make comparisons of the dissections with Fig. 1 in order to discover for yourself the structural plan of body regions and the vessels draining them and the general course and interrelations of the vessels themselves.

Veins Entering the Sinus Venosus.—The *right precava* is a large vessel opening into the right side of the sinus venosus. Its branches return blood from the right side of the head, the right forelimb, and the skin of the right side of the back. Gently take hold of the tip of the ventricle with the forceps, raise the heart, turn it to the frog's left, and observe the short precava lying close to the auricle. Follow this short vessel latero-cephalad from the heart to a point just outside the peritoneal membrane where it is formed by three tributaries:

a. The *external jugular* is the inner of these three divisions and drains the floor of the mouth and the tongue. Close to the ventral surface of each external jugular vein is a small, round, highly vascular body, the *thyroid gland.*

b. The middle of the three tributaries of the precava is the *innominate vein*, coming from a lateral direction and earlier from abruptly dorsad, receiving as it turns a small vein from the back of the arm and shoulder. The main vein before the entrance of

this branch is the *internal jugular,* returning blood from the interior of the skull, which it leaves by an aperture at the posterior border of the orbit.

c. The third and outer tributary of the precava is the *subclavian* vein. It is the largest of the three and comes from the body wall to the base of the arm where it receives the *brachial* vein from that appendage. Before this point it is found as the large *musculo-cutaneous* vein which returns blood from the skin and muscles of the side and back of the body and from the head.

The *left precava* in its course and tributaries corresponds to the right precava. It may be located.

In synoptic fashion (or otherwise), show: (*a*) the region drained by the precava, (*b*) the principal tributaries of the precava and the areas drained by each.

The *postcava* is the only vein returning blood from the posterior part of the body. It drains the kidneys and liver and through them the remaining organs. Expose both kidneys ventrally by removing the peritoneum and pushing back the other structures which obscure them. Note how the postcava is formed between the kidneys by the *renal veins* and follow it cephalad to the liver. Dissect away enough of the substance on the dorsal side of the left lobe of the liver to show that the postcava passes through this organ without breaking up into capillaries. As it emerges from the liver under the apex of the heart it receives the right and left *hepatic veins* and passes directly into the sinus venosus. Harmonize this dissection with the structural principle shown in Fig. 1 and relevant statements already made.

Veins Entering the Left Auricle.—The *pulmonary vein* is formed by the union of the right and left pulmonary veins and returns blood to the heart from the lungs. Each pulmonary vein extends along the inner side of the lung and may be located by dissecting away, little by little, the membrane between the heart and the lungs.

The Portal Systems.—The liver is one of the organs in the frog's body in which veins en route to the heart break up into capillaries and then reunite to form veins before leaving the organ. Because of this peculiarity of structure this portion of the venous system is known as the *hepatic portal system.* There is another portal system in the frog which is located in the kidneys and called the *renal portal system.* The veins which carry blood to

those organs are called *portal veins* and are distinguished by prefixing the name of the organ.

The Renal Portal System.—Remove the skin from the dorsal surface of the left thigh, separate the muscles by tearing the connective tissue between them. Between the two larger muscles will be found a smaller one which may be transected at its middle. Underneath this small muscle can be seen the *femoral* vein which drains the hind limb. Remove such muscles and portions of the skin and body wall as are necessary to trace this vein to a point on the posterior wall of the body cavity where it forks giving off a dorsal branch, the *renal portal* vein, and a ventral branch, the pelvic vein. Tear the peritoneum along the outer margin of the kidney so as to expose this organ. Now trace the *left renal portal* vein along the outer margin of the kidney and note that it breaks up into capillaries within this organ. Through what vessels does the blood emerge from the kidneys, and by what main trunk does it reach the heart? The *right renal portal* corresponds in its course and branches to the left one. Uncover this vessel and also the right.

The Hepatic Portal System.—The union of the two *pelvic* veins, one from each side of the body, forms the *ventral abdominal* or the vein which was earlier dissected from the ventral body wall. The hepatic portal system is formed partly by the ventral abdominal vein, which brings to the liver blood from the hind limbs; and partly by veins returning blood from the alimentary canal and its appendages. Trace the *ventral abdominal* vein cephalad to the liver and note that it divides into right and left branches, which enter the right and left lobes of the liver, respectively. The *hepatic portal* vein is a large vein which runs in the mesentery and joins the ventral abdominal vein at its point of division into right and left branches, giving off, before doing so, a branch to the left lobe of the liver. It carries to the liver the blood from the walls of the alimentary canal and is formed by the union of the (1) *gastric veins*, from the stomach, the (2) *intestinal* veins, from the whole length of the intestine both small and large, and (3) the *splenic* vein, from the spleen. This usually joins one of the intestinal veins.

Reduce this dissection to the simple principle of "region drainage" illustrated in Fig. 1.

On the plate showing outlines of some of the visceral organs, indicate the course of one of the precaval veins and its tributaries

and label. Do the same for the pulmonary veins. Represent the postcava and its tributaries, the renal portal system of the left side, and the hepatic portal system. Label parts of each.

Review the blood system by comparing the schematic representation of Fig. 1 and the labeled plates.

THE NERVOUS SYSTEM

In an earlier study it has been demonstrated that the contraction of muscles when properly attached to skeletal elements may cause parts to move. Before a muscle contracts it receives a nervous stimulus from a controlling center, and before complicated and beneficial movements take place these muscular contractions must be coordinated. If an animal is to acquire any degree of intelligence it must be able to accumulate experiences. These may be taken as the general services provided by the nervous system.

This communicating, coordinating, and controlling system in animals like the frog and man comprises two divisions: (1) the central *nervous system*, formed by the *brain* and *spinal cord;* (2) the *peripheral nervous system*, comprising the nerves which extend between the central system and the periphery of the body (or outlying organs). Specimens for this study have been prepared so as to soften the bones which may be cut with instruments.

If the skin has not already been removed from the animal clip off that of the dorsum. Remove a narrow strip of muscle from the middorsal region beginning at the cranium and extending to the sacral vertebra. Expose the brain and spinal cord by carefully snipping away the roof of the skull and vertebral column with the aid of scalpel, forceps, and scissors. Begin by cutting through the cartilage just caudad of the external nares from this point work caudad slicing off the dorsal portion of the cranium and vertebral column until the outlines of the brain and spinal cord are exposed to view. Care must be used to avoid cutting or injuring the soft nervous tissues while removing the roof of the skull and vertebral column and to avoid injury to the nerves leaving any part of the central system. Do not cut away too much of the neural arches of the vertebræ. In removing pieces of skeletal material from the sides of the central nervous system always cut away from the middorsal line. When the central nervous system is clearly exposed identify the parts to be seen from the dorsal aspect.

Dorsal Aspect of the Brain.—Observe the pigmented membrane closely applied to the nervous tissue. This membrane is called

the *pia*. In all probability the outer menix (covering or envelope), the *dura*, has been more or less torn in removing the bone, since in the frog it appears more as a lining of the cranial cavity than as an envelope of the brain. The pigmented state of the pia is especially noticeable in the caudal portion of the brain, where it is rich in blood vessels and roofs over a triangular cavity. With the forceps or needles remove the pia.

For descriptive purposes the brain will be regarded as comprising divisions and parts as follows:

1. The *forebrain* exhibiting two divisions. The more cephalic portion consists of a pair of rounded *olfactory lobes*, which are separated by a faint mesal groove and are continued cephalad as the "olfactory nerves" which extend to the organs of smell located in the nasal canal. The caudal boundary of the olfactory lobes is marked by a slight transverse depression, behind which is to be seen the other portion of the forebrain, namely, a pair of long oval bodies, the forebrain *hemispheres*.

2. Just caudad of the hemispheres is a constricted region, the *twixt brain* or *interbrain*, bearing in the midline and near the caudal end of this region a short stalk, the *pineal body*. In the undisturbed state this is found to extend to the bony roof of the cranium where it ends in a knoblike enlargement. In the tadpole (young frog) this outgrowth appears to be connected with that area already identified on the dorsum of the head as the *brown spot* which is looked upon as marking the location of a former sense organ (or organs). The pineal stalk of the brain represents its former nervous connections.

3. Caudad of the interbrain is the *midbrain* of which the *optic lobes* are the prominent features of the dorsal aspect.

4. Just behind the optic lobes is a narrow, transverse ridge, the *cerebellum*. This division in many other animals is larger and an important center of equilibrium. In the frog it is not extensively developed.

5. The division caudad of the cerebellum is the *oblongata* or *medulla oblongata*. Its dorsal wall is a thin, vascular membrane consisting of pia and endyma (the lining of the cavity of the brain). In the roof of this division nervous substance disappears. The vascular membrane thus formed is known as the *posterior choroid plexus*.

The Spinal Cord.—The spinal cord is continuous with the oblongata and occupies a space within the vertebral column

known as the *neural canal* formed by the neural arches extending dorsally from the centra of the vertebræ. In the mesal line is a longitudinal groove, the *dorsal fissure*. Observe the cephalic or *brachial* and the caudal or *lumbar* enlargements of the cord. Caudad of the lumbar enlargements the cord is reduced to a mere filament, the *filum terminale*, which extends into the cavity of the urostyle and may be seen by dissecting away the dorsal portion of this part of the skeleton.

The Cranial Nerves.—Ten pairs of nerves arise from the brain within the cranial cavity, hence their name. Some are sensory, others motor, and some are both sensory and motor in nature. The roots of some of these may be seen if care is used in making the dissections necessary to expose the sides of the brain. A brief description of these nerves is given as an aid to the identification of those which may be found without the expenditure of too much time. *If time is limited proceed directly to the study of the brain cavities.* The cranial nerves are in pairs, one leaving the brain on each side. The first is the *olfactory* and has already been seen, arising from the olfactory lobe. The second, or *optic nerve*, leaves the ventral surface of the interbrain and extends directly to the eye. The third, the *oculomotor*, is small and arises caudad of the optic nerve and is distributed to muscles of the eye. The fourth, the *trochlearis*, is very small and leaves the brain from the dorsal side between the optic lobe and the cerebellum. Both the third and fourth nerves are motor and are distributed to certain muscles of the eyeball. The fifth, the *trigeminal*, is one of the largest of the cranial nerves. It leaves the ventral side at the cephalic end of the oblongata. It is distributed to the side of the head and lower jaw. The sixth, the *abducens*, arises from the ventral side of the oblongata and supplies certain eye muscles. The seventh, the *facial*, arises from the oblongata caudad of the fifth and is a mixed nerve to the side of the head and lower jaw. The eighth, the *auditory*, arises from the dorso-lateral wall of the oblongata and supplies the inner ear. The ninth and tenth, the *glossopharyngeal* and *vagus*, respectively, arise together from the sides of the oblongata a short distance caudad of the eighth. Both contain sensory and motor fibers.

Both brain and spinal cord are hollow, the cavities of the two being continuous. Carefully slice away the dorsal surface of the brain in order to expose its cavities called *ventricles*, and if

time permits proceed to identify the cavities mentioned in the following lines. The cavities of the forebrain hemispheres are called *lateral ventricles.* The cavity of the interbrain is the *third ventricle,* that of the oblongata region the *fourth ventricle.* The narrow canal between the third and fourth ventricles is called the *aqueduct of sylvius.*

The Spinal Nerves.—In order to study these nerves, make a midventral incision through the body wall from the pelvic girdle to the cephalic margin of the pectoral girdle and lateral incisions through the body wall just cephalad of the pelvic girdle. Remove all of the viscera in a mass by cutting across the organs at the cloaca and loosening them from the vertebral column with a scalpel, lifting them as the necessary cuts are made. When they are completely freed from the vertebral column, sever the mass in the region of the œsophagus. The spinal nerves will now be seen as white threads lying on the dorsal body wall.

Note in the arm and leg regions the grouping of the spinal nerves into plexuses. The *brachial plexus* is formed by the mingling of the fibers of the *second* and *third* nerves. It gives rise on either side to the *brachial nerve,* which is the important supply to the arm. The *sciatic plexus* is similarly formed from the *seventh, eighth,* and *ninth* nerves and gives rise on each side to the *sciatic nerve,* the main nerve of the leg. The *first spinal nerve* is small and arises just cephalad of the brachial plexus to supply the muscles of the back and shoulder.

The *fourth, fifth,* and *sixth* nerves are also small and supply the muscles and skin of the body wall. The small *tenth nerve* issues from the urostyle and sends out branches to the bladder and cloacal region.

Select one of the large nerves making up the sciatic plexus and trace it to the point where it leaves the vertebral column, carefully cutting away the vertebræ on the dorsal side. Each of the ten pairs of spinal nerves arises from the cord and leaves the neural canal by way of openings between the vertebræ. Each spinal nerve has two roots, a *dorsal* and a *ventral.* The dorsal root is known as a *sensory* root, since it is composed of sensory fibers and conducts impulses toward the cord (afferent impulses). The ventral root is termed the *motor* root, because it is composed of motor fibers which transmit nerve impulses away from the cord (efferent impulses). The two roots unite to form a common trunk outside the neural canal. Trace the roots further toward

the cord and note that the dorsal root is attached to the dorso-lateral and the ventral root to the ventro-lateral region of the cord. A small *ganglion* (enlargement) lying in the intervertebral foramen (space between two adjoining vertebræ) and surrounded by the white *periganglionic gland* (the calcareous body) is found on the dorsal root near its union with the ventral root, but in the frog as a rule the spinal ganglion is continued beyond the point of union so that the two roots appear to meet in the ganglion itself. Examine the periganglionic gland and then remove it so as to reveal the small brown *spinal ganglion*.

Label the described parts on the outline of the dorsal aspect of the brain and spinal cord.

SYNOPTIC SUMMARY OF THE DIVISIONS OF THE NERVOUS SYSTEM

- Nervous system
  - Central system
    - Brain
      - Forebrain
        - Olfactory lobes
        - Forebrain hemispheres
        - Lateral ventricles
      - Interbrain
        - Pineal body
        - Pituitary body
        - Infundibulum
        - Third ventricle
      - Midbrain
        - Optic lobes
        - Aqueduct of sylvius
      - Hindbrain
        - Cerebellum
        - Oblongata
        - Fourth ventricle
    - Spinal cord
  - Peripheral system
    - Cranial nerves
      - I. Olfactory
      - II. Optic
      - III. Oculomotor
      - IV. Trochlearis
      - V. Trigeminus
      - VI. Abducens
      - VII. Facial
      - VIII. Auditory
      - IX. Glossopharyngeal
      - X. Vagus
    - Spinal nerves 1 to 10
    - Sympathetic system. Not included in this study

THE COMPOUND MICROSCOPE AND ITS USE

In order that one may be able to make the best and safest use of so complex, delicate, and expensive an instrument as a compound microscope a limited amount of time is set aside for the study of its construction, care, and use.

Possibly all will profit somewhat by a careful examination of the instrument which is to be used during the course. The following instructions are primarily for beginners, but all are

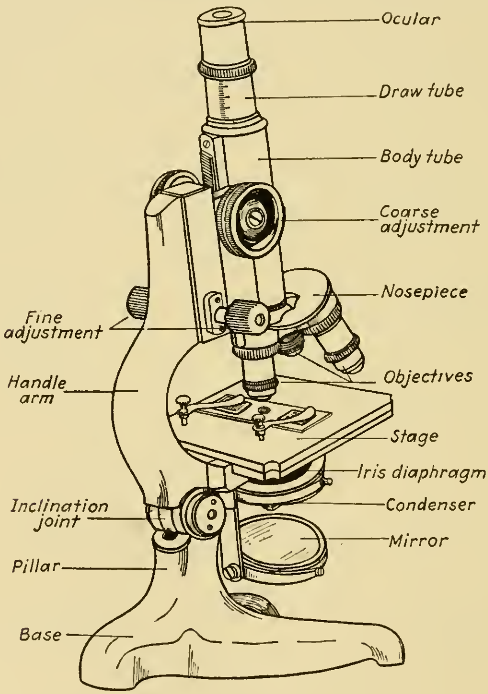

Fig. 2.

asked to read them and perform the various manipulations which are mentioned. As those who have never used a microscope will require extra time for the study of its parts and especially for the practice which is necessary to the effective use of the instru-

ment, those who are proficient in its use may turn immediately to the study of the cellular structure of the frog. *No one should attempt to study with the microscope before* being able first to focus on an object with the low-power objective and then change to the high power and find the object without allowing the high objective to come in contact with the cover slip on the slide. *Touch only metal parts.* Consult Fig. 2 of a compound microscope and identify the base, pillar, stage, handle arm, body tube, coarse and fine adjustments, nosepiece, objectives, oculars, iris diaphragm, and mirror.

In using the instrument, light is reflected from the movable mirror through the aperture of the diaphragm in the stage, upon which is placed the object to be examined. The observer looks through the body tube to which the ocular and objectives are attached. The revolving nosepiece at the lower end of the tube holds the objectives of different powers and facilitates the change from one to the other by turning upon its axis. It is, therefore, *unnecessary* to remove an objective from its attachment to the nosepieces.

Always work with the low-power objective and ocular first. These will soon be learned by the size and number. Place a prepared slide of a wing of the house fly upon the stage, center the light with the mirror, and by means of the coarse adjustment bring the low-power objective one-fourth inch above the slide. Then, looking into the ocular, focus the microscope by gradually raising the lens, using the coarse adjustment, and turning counterclockwise with the right hand until the object is brought into view. The focus may then be sharpened by the use of the fine adjustment. Compare the orientation of object and image.

Note carefully the brightness of the field of vision and the appearance of the object; it is illuminated by transmitted light, *i.e.*, light which is reflected from the mirror and passes through the object. Tilt or cover the mirror and observe the change in the intensity and character of the light. The object is now viewed by reflected light, *i.e.*, light which is reflected from the surface of the object. Reflected light must be employed for all opaque objects.

In order to use the high-power objective, first place the slide so that the object appears exactly in the center of the field as viewed with the low-power objective; then increase the light and change to the high-power objective by revolving the nosepiece

until the objective is in line with the tube. Its exact position is indicated by the "click" of a friction stop. The object can then be seen dimly. To sharpen its outlines employ the fine adjustment turning only slightly to the right or left. It is always safest to *focus up* slightly before changing the objective, thus avoiding the danger of the high objective coming in contact with the object. Use *great care* in changing from low to high power.

Thoughtful practice in the use of the compound microscope soon results in effectiveness on the operator's part. *Always observe the following rules* in using a compound microscope:

1. Do not touch the glass of the objectives and oculars with the fingers or any object. If the surfaces become soiled ask the instructor's aid in cleaning them with lens paper.

2. It is dangerous to both lens and object to focus downward with the eye on ocular.

3. Never permit the objective to touch the cover glass.

4. Do not use high powers when low ones will do.

5. Always carry your instrument by the handle arm. Otherwise the adjustments may be injured.

6. Do not leave mirrors or lenses exposed to direct sunlight.

7. In case of trouble consult your instructor. When the microscope does not appear to behave properly, it is safe to assume that the trouble resides in the operator rather than in the instrument.

THE FINE STRUCTURE OF THE FROG

In the previous laboratory studies of the frog the gross structure only has been emphasized. The body was found to be composed of organs associated as systems of organs each with a particular service to perform. When a small portion of any organ is examined microscopically it is found to be composed of great numbers of units called *cells*. Each cell is a minute mass of protoplasm. When more extensive microscopic studies of the body are made two configurations are readily recognized:

1. The protoplasm of the body rather than existing as a mass is everywhere divisioned as cells. The body is therefore to be regarded as formed of cells and is said to be *multicellular* in its structural nature.

2. The cells are not all alike. Different groups of cells while agreeing among themselves differ from those of other groups and each different group is concerned with different categories of functions. Each of these groups constitutes a *tissue* which may be defined as an association of cells differentiated in relation to a particular category of functions. There are four primary tissues as enumerated below.

a. Epithelium or the covering, protecting and secreting tissue. Normally nowhere in the body is any other kind of tissue exposed. Not only the external surface but also the lining of every cavity is composed of this kind of tissue. Epithelial tissues constitute the active elements of glands.

b. Connective tissue, as the name suggests, functions as the connecting and supporting substance of the body. It fills spaces in the body, unites the various parts of the body, and binds organs together.

c. Muscular tissue is the tissue of movement.

d. Nervous tissue comprises elements adapted to the conduction of impulses and serving as the communicating lines in the body.

When a cell is studied microscopically it is found to be composed *not* of a homogeneous substance but of organized protoplasm.

A STUDY OF THE PRIMARY TISSUES

Epithelium.—One of the most important functions of epithelium is protection or the covering of other tissues. An example of such an association of cells is the pavement-like layer of flattened cells which constitute the outermost layer of the skin. Examine a mounted preparation of such a layer which may be prepared from the sloughed skin of the frog. This shows the skin as it actually appears in surface view. Note that the cells are all similar in appearance and so associated with one another as to protract a sheet of cells which is the tissue. In studying tissues it is always best to use a minimum of light. Tilt the mirror of the microscope or reduce the opening in the diaphragm in order to reduce the amount of light passing through the preparation.

Study a single cell and determine the parts which are common to all cells, as follows: (*a*) *cell membrane,* the thin but dense margin of the cell; (*b*) *nucleus,* composed of dense protoplasm and located near the center of the cell; (*c*) all the protoplasm excluding the nucleus is termed the *cytoplasm.* Each of these parts and many others not mentioned is an area of protoplasm differently organized for particular services in the cell.

Make an enlarged drawing of several cells showing their relations and parts. Drawings of single cells should not be less than three-quarters of an inch in diameter.

Many adaptations to various functions are illustrated by epithelial tissues and in each case the tissue takes a name descriptive of its form, structure, or function. For instance, in some regions, as in the lining of the mouth and œsophagus of the frog, the exposed surface of the epithelial cells is provided with delicate, vibratile extensions of protoplasm called *cilia* and it is accordingly known as a *ciliated epithelium.* Examine a strip of the lining from the roof of the frog's mouth stretched and moistened with normal salt solution to demonstrate the transportation of solid particles such as fine charcoal over the surface of the epithelium by the effective stroke of the cilia. Examine a piece of teased epithelium as a demonstration of ciliary action.

Connective tissue performs varied functions and is correspondingly modified in its nature. White fibrous connective tissue, yellow elastic fibers, adipose tissue, cartilage, and bone are here considered as forms of connective tissue. While in some tissues the intercellular substance is relatively small in

amount, in connective tissue it is usually abundant. Connective tissue is composed of scattered cells, together with the intercellular substances. The intercellular substances vary according to the service performed by the tissue.

As an illustration of the binding and connecting function of this primary tissue study preparations made from materials taken from between the larger muscles of the frog. The cells themselves are widely separated by the fibrous intercellular substance which they secrete. These fibers are of two kinds: (*a*) fine ones arranged in wavy bundles known as *white fibers* and (*b*) *elastic fibers*, which are larger, single, and approximately straight in their course.

Make a drawing of a restricted area to show the characteristics of this type of tissue. Label the parts and point out the correlation of intercellular substance and the connecting function.

Cartilage serves as an example of connective tissue in which the intercellular substance is firm, adapting it for the purpose of meeting the needs of stress, strain, and impact. Examine a prepared section of the cartilaginous portion of the sternum of the frog. Note that it is composed of a non-cellular homogeneous *matrix* within which the cells are scattered in rounded spaces or *lacunæ*. Each cell secretes about it the intercellular substance or matrix. Around the border of the cartilage may be seen a connective tissue layer containing cells, which, as the cartilage increases in mass, become transformed into functioning cartilage cells and surrounded by matrix. Keep in mind that the functional part of cartilage is the matrix.

Make an enlarged drawing of a small area of cartilage and label the parts.

Muscular tissue is composed of elongate, contractile cells bound together by connective tissue. Muscle cell and muscle fiber are synonymous terms. The structure of two types of muscles, *striated* and *non-striated*, will be studied.

Striated Muscle.—Other names for this type of muscle may be recalled from the directions on the muscular system. Examine a prepared slide of muscle fibers. First locate the material with the low-power objective and then study it under the high power. The long and relatively narrow elements are portions of muscle cells or fibers, their large size obviating the possibility of securing sections of a complete cell. The ends therefore appear broken. Every skeletal muscle is composed of a large number of fibers or

cells. Identify the delicate membrane or *sarcolemma* which covers each fiber. This thin covering is best seen in places where the fibers are crushed or broken apart. Each fiber shows both a longitudinal and a cross striation. The longitudinal striation is due to minute strands, the *fibrillæ*, which extend the length of the fiber and are bound together in the same sarcolemma sheath. The transverse striations are due to alternately dark and light bands in the fibrils. Each muscle cell also contains a number of spindle-shaped nuclei scattered among the fibrils. The young muscle cell possesses but a single nucleus which during growth divides repeatedly, resulting in the multinucleate condition.

Make a drawing which will show all the structures listed above.

Non-striated Muscle.—Study a stained preparation. Non-striated muscle tissue is composed of long, slender, spindle-shaped cells arranged parallel to each other. Each long fiber or cell possesses an oval nucleus near its center.

Make a drawing of a single fiber, showing parts described.

Nervous Tissue.—A nerve cell, or *neuron*, consists of a central portion, called the *cell body*, which contains a large nucleus, and of slender processes which extend out from this cell body often to great distances. Portions of nervous tissue which consist predominantly of cell bodies are spoken of as *gray matter*, while those which consist of the processes constitute the *white matter*. The processes of a nerve cell are of two kinds: those which convey the impulses toward the cell body, called *dendrites*, usually very numerous and much branched, and those which convey the impulse away from the cell body, usually single and unbranched or slightly branched, called the *neurite* or *axone*. What one refers to as *nerves* are bundles of these processes. Histologically, nervous tissue consists of (1) the nervous matter proper, *i.e.*, nerve fibers or neurones, and (2) delicate supporting and connective cells called *neuroglia* cells, in which the nerve cells are embedded.

Study a cross-section of the spinal cord of the frog. Under low power identify the narrow *dorsal fissure* and the broader *ventral fissure*. Notice that the cord differs in appearance in different parts. There are two general regions, a central, slightly darker region, the *gray matter*, trapezoidal in shape and containing numerous darkly stained cell bodies; and an outer, lighter region, the *white matter*, with only a few small cell bodies. The

corners of the trapezoid of gray matter extend dorsad and ventrad, forming the so-called *dorsal* and *ventral horns* of gray matter The larger cell bodies seen in the ventral horns are those of the *motor cells* whose axones extend out to the voluntary muscles or other effectors of the body following a course through the ventral root of a spinal nerve. Examine one of these under high power. The structure of the cell body is well shown but only the beginnings of the processes are present, as these have been cut across in preparing the thin sections. Note the relatively large *nucleus* of the cell body containing a conspicuous round body, the *nucleolus.* Often a more deeply staining group of cells may be seen in the center of the cross-section, the epithelial cells lining the *cavity of the cord.*

The general structure of nervous tissue may be determined by the study of prepared slides of teased and stained tissue.

Sketch a cross-section of the cord as it appears under low power (about two and one-half inches in diameter), stippling the grey matter more heavily than the white matter. Do not attempt to show cellular structure in the cross-section but draw a single motor cell body separately as seen under high power.

MITOSIS

As the cellular nature of the fundamental tissues found in the bodies of higher animals becomes apparent there naturally arises the question of how these tissues increase in amount or bulk to account for the increase in the size of the animal during growth. Since the cells of any given tissue do not increase in size beyond a certain maximum it seems reasonable to accept the explanation that increase in the size of an animal is due to an increase in the number of component cells. The manner of cell increase is that of cell division. A given cell at the proper time divides into two daughter cells which grow to the normal size and in their turn divide. In most tissues the method is that known as *indirect cell division* or *mitosis*.

Growing tissues of almost any organism might be used to illustrate indirect cell division, but for several reasons the developing eggs of *Ascaris megalocephala*, a parasitic roundworm, are recommended.

In the female Ascaris the reproductive organs are so modified as to comprise various specialized parts one of which is the *uterus* or part retaining the eggs during development. Sections through the appropriate portion of the uterus show the eggs (cells) in various stages of mitosis. Such sections should be mounted in rows lengthwise of the microscopic slide. A well-established method is that of arranging sections showing maturation, fertilization, and mitosis in five rows across the slide, the fifth or bottom row containing cells in a state of active mitosis. Whatever the arrangement it should be explained by the instructor by way of saving time for actual study.

If Ascaris material is used, examine the section of the fifth row on the slide with the low power of the compound miscroscope and identify the thin walls of the uterus composed of large epithelial cells and its wide cavity completely filled with circular objects, each of which is a section of an egg or true cell. Examine one of the eggs under high power and get a clear idea of its structure. Identify the thick shell or capsule inclosing a cavity occupied by the egg cell which is considerably smaller than the

cavity itself. The cytoplasm of the egg appears vacuolated, that is, appears to contain empty spaces. Dark-staining bodies, the chromosomes, will be seen in the nucleus. Search the cells for typical examples of the stages in mitotic cell division as called for below.

It may not prove an easy task to find just the stage desired, but only as a last resort should assistance be requested of the instructor. The difficulty lies in the fact that the sections are very thin and therefore a complete picture of what is desired may not be found in a given section unless it passes through the proper plane of the cell. Avoid the study and drawing of such partial figures and represent egg cells at least an inch and a half in diameter.

In the following paragraphs the stages or phases of mitosis are arranged in the order in which they occur. Although phases are referred to for descriptive purposes it is to be kept in mind that mitosis is a continuous process and that the stages here described overlap in varying degrees. The term *phase* or *stage* is employed as a name for the completion of certain events regardless of whether these events are completed before or after another begins.

Early Prophase Stage.—As cell division begins, the chromatin substance of the nucleus appears to form a long, coiled thread. Consider that in sections only pieces of such a coiled thread could appear and then note in the demonstration of this stage the deeply stained, elongate pieces of chromatin to be seen in the nucleus.

Late Prophase.—The thread is resolved into a number of distinct slender bodies, each of which is called a *chromosome*. In Ascaris there are four of these. The small number of chromosomes is one of the advantages of Ascaris material for this study rather than cells from the frog where 24 chromosomes appear. In well-prepared material there will be observed in the cytoplasm close to the nucleus two dense areas of granules called the *asters*. Each aster contains a dark center, the *centrosome*, associated with radiating fibrils, the *astral rays*, reaching out into the surrounding cytoplasm. Between the centrosomes, which come to be located at opposite poles of the cell, there extend similar delicate fibrils which collectively are called the *spindle*. The spindle together with two asters constitutes what is known as the *achromatic figure*, or *amphiaster*. Sometime during the processes

just described, a splitting of the chromatin threads has occurred. These split threads or chromosomes appear to shorten and thicken. The nuclear membrane disappears leaving the chromosomes free in the cytoplasm. The chromosomes now appear in pairs, due to splitting, and become arranged upon the spindle in a plane midway between the two centrosomes. This plane is referred to as the *equatorial plane.* The series of events culminating in this manner is said to constitute a stage in mitosis known as the *prophase* or the preparation stage for actual division.

Metaphase.—Following late prophase there appears to be a short period during which the chromosomes are arranged in the equatorial plane. It is a state of balance. If the chromosomes have not already split they do so at this time.

Find a cell cut across the middle of the spindle (in an equatorial plane) and showing the four split chromosomes free in the cytoplasm. Centrosomes and astral rays will not show in such a section. Draw.

Locate a cell which is cut through the axis of the spindle (in a meridional plane). This should show centrosomes, astral ray, and spindle with the band of chromosomes across the middle of the mitotic figure. Draw.

Anaphase.—One of each pair of the split chromosomes moves toward its respective centrosome or aster. In this migration the ends of the chromosomes always point toward the axis of the spindle, so that the cell contains two groups of chromosomes, each group looking somewhat like an open umbrella with the delicate threads of the spindle stretching between them. Draw.

Telophase.—The chromosomes approach the asters, where each group condenses into a mass in which the identity of the chromosomes is lost. A constriction in the cytoplasm gradually deepens and divides the cell in two. Draw. The chromatin mass of each daughter cell reorganizes a nucleus with a nuclear membrane. Two daughter cells, believed to be exactly like the original mother cell and like one another, have thus been produced.

A STUDY OF THE BASIC REPRODUCTIVE AND HEREDITY PHENOMENA

Maturation and Fertilization.—For this study the eggs of Ascaris are described, but taken from another part of the uterus than that supplying the cells for the study of mitosis. Sections of such eggs may be mounted as the first four rows upon a slide with sections arranged according to the method mentioned above.

It will be profitable to recall from lecture and textbook studies what is meant by the terms *somatic cells*, *germ cells*, *gametes*, *microgamete*, *macrogamete*, *ovum*, *spermatazoon*, and *zygote*. It is also important to keep in mind that a new individual comes into existence through the fertilization and development of an egg, which means that: An egg (ovum or macrogamete) fuses with a zoosperm (microgamete) which constitutes the beginning of a new individual. That is, two independent cells fuse to form a single cell (fertilized egg or zygote), which is a new individual.

The cells in the body of each species possess a definite and constant number of chromosomes. Since two cells fuse in the creation of a new individual it ordinarily would follow that with each fertilization the chromosomes would double in number in each new generation. This does not occur, as is known from observation. It follows, then, that as regards the gametes some provision is made whereby the normal number of chromosomes is maintained. The object of the present study is that of demonstrating the way in which this comes about. The process is known as the *maturation* of the germ cells or their preparation for the fertilizing process.

In this species of Ascaris maturation of the egg follows the *entrance of the sperm*. The sperm meets and enters the cytoplasm of the egg which is still naked. Soon after, the walls of the uterus secrete an albuminous covering about the egg and two maturation divisions of the egg nucleus take place before it is ready to fuse with the sperm nucleus, thus completing the formation of the zygote.

Entrance ofthe Spermatozo on into the Egg.—Examine enough eggs of row 1 to get an idea of the form of the spermatazoon and its

position in the cytoplasm of the egg. Note the head containing the round *nucleus* and the more pointed portion termed the *refractive body.* Also the lack of an enclosing capsule about the egg. In some of the eggs the refractive body of the sperm is probably being absorbed. Draw.

First Maturation Division of the Egg (Row 2).—This is known as *reductional division,* as the number of chromosomes of the egg are reduced by one-half so that the number of chromosomes peculiar to the body cells of the species remain constant after union with the sperm nucleus (pronucleus) which has undergone reductional division of its chromosomes before entering the egg. Find an egg showing a spindle upon which are two sets of chromosomes. One set will be separated off and thrown out of the cytoplasm of the egg as a small discarded cell known as the *first polar body,* the other set will be retained as those of the *egg nucleus.* The *sperm pronucleus* ought to be at rest somewhere within the cytoplasm. Draw.

Second Maturation Division of the Egg (Row 3).—This is known as *equational division,* as the number of chromosomes of the egg nucleus remains the same thereafter and is a true mitotic division resulting in a second polar body which is cast off and, like the first, comes to naught. In a typical example of this stage the *first polar body* ought to be seen clinging to the inside of the egg capsule, the chromosomes of the *second polar body* on a spindle with those of the egg mucleus, and the *sperm pronucleus* at rest somewhere in the cytoplasm of the egg. Note that the cytoplasm of the egg has withdrawn from the egg capsule leaving a space between the two. The cell remaining after the separation of the two polar bodies becomes the functional egg. Draw.

Stage Preceding the Union of Male and Female Pronuclei (Row 4).—This might be compared with the resting stage during mitotic division of somatic cells, since mitotic division follows the union of the male and female pronuclei. Look for a cell which shows: (*a*) *two nuclei,* one the *male,* the other the *female,* but indistinguishable from one another; (*b*) the *first polar body* within the egg capsule, and the *second polar body* clinging to the outer margin of the cytoplasm of the egg. Draw.

A STUDY IN DEVELOPMENT

The object of this study is that of illustrating the manner in which a multicellular animal is derived from a single cell. All

multicellular animals which reproduce according to the gamic method (sexually) begin their existence as a single cell, the zygote. After fertilization (syngamy) or the union of the two gametes the resulting zygote (single cell) undergoes mitotic division. In the division of the zygote this is referred to as the cleavage or segmentation of the egg. The resulting cells remain associated in a cluster, and thus the multicellular state of the new individual is attained.

These cleavage or segmentation stages, which are preliminary to the building up of the tissues and organs of the new individual, can better be studied in such an egg as that of the starfish, in which segmentation is equal and complete, than in the egg of the frog, in which segmentation is complete but unequal. The principle, however, is the same in both.

Development of the Starfish (Asterias).—Slides should be furnished which contain at least seven stages in the ontogeny (development of the individual) of this animal. Use *low power only* and locate the unfertilized egg which can be recognized by its large clear *nucleus*, containing a black spot, the *nucleolus*. As it is often difficult to find such small eggs on a slide, it is suggested that one focus with the low power on the edge of the cover slip. Then when the slide is moved so as to bring an egg under the objective it will be seen. Having found this initial stage in the development move the slide and with the aid of the following descriptions identify and study each of the stages:

1. The unfertilized egg represents a single cell. The cytoplasm and nucleus with its small nucleolus should show distinctly; also the egg membrane (or vitelline membrane). The colors are due to the dyes used in staining the material in preparation for study.

2. After fertilization the egg divides by mitosis. The first division, the plane of which is meridional in direction, gives rise to the two-celled stage, the two daughter cells remaining in contact with each other, and the *fertilization membrane* may be seen enveloping both cells.

3. The second division occurs at right angles to the first but also meridional in direction, resulting in the four-celled stage.

4. The third cleavage plane appears at right angles to the first two and may be spoken of as equatorial in position. Eight equal cells result.

5. The process of segmentation continues until a large number of cells is produced. Some slides may show the 16- or 32-cell stages. Meanwhile a central cavity, the *segmentation cavity*, forms, about which as a wall the cells are arranged.

6. A continuation of the process of cleavage results in the formation of the coarse morula stage, and ultimately there is reached the fine morula or *blastula* stage in which the single layer of cells surrounding the segmentation cavity persists. These stages are in the form of a hollow sphere but may be deceiving in some preparations, as the walls often collapse. Carefully focus on one of these balls until the appearance is the same as if a section had actually been made through the center of the blastula. The blastula then appears as a hollow hemisphere of cells, the layer being one cell thick, surrounding the large cavity. Such a view is termed an *optical section.*

7. Cell division continues but the cells begin to show their differentiation and shift in position. The cells in one half of the hollow sphere come by invagination to lie within those of the other half. It is analagous to pushing in one side of a hollow rubber ball. Thus an embryo is formed with a body wall of two layers of cells. Study as in optical section. The outer layer of cells is called the *ectoderm;* the inner layer, which has invaginated, the *entoderm;* the space resulting from invagination is the *archenteron* or primitive intestine; and the opening of the archenteron to the outside is the *blastopore.* This stage is called the *gastrula* since the alimentary canal is here first formed. The next stage in development is the formation of a third layer of cells, the *mesoderm,* between the other two. These three layers are called *germ layers* and from them all of the tissues and organs of the body are derived.

On the plate showing some of the early stages in the development of the starfish, first give a proper heading to the plate, then supply the appropriate title for each figure and label all structures discussed in the outline.

Development of the Frog.—The common leopard frog lays its eggs during April and May in the shallow water of ponds and the margins of lakes. All the eggs are laid at one time in a single complement, each egg being surrounded by a gelatinous envelope.

After fertilization by the spermatozoa of the male, which are cast over the eggs as they are extruded by the female, cleavage

follows and the developmental stages already noted in the study of the starfish are completed in regular order, resulting in a multicellular body with all the necessary organs. When the eggs hatch, the young animals are not in the form nor do they possess the habits of the adult. They enter upon a true *larval stage* which may be viewed as one occurring between the egg and adult stages for the completion of development. During the larval period the structure and functions of the body are such as conform to its aquatic life, necessitating many organs such as gills and a swimming tail not present in the adult which leads a terrestrial life. At the end of the larval period the larval structures are lost and those of the adult are assumed.

Due to the presence of a large quantity of yolk in the frog's egg and for other reasons, the details of development vary from those found to obtain in the starfish. When properly viewed, however, they are found to be fundamentally alike.

Stages illustrating the early development of the frog may be studied from preserved material.

By pouring the liquid containing this material into a watch glass or other container the several stages may be studied with a hand lens or, better, with a dissecting microscope. Small camel's-hair brushes are convenient for moving the material in the liquid. Care must be used not to destroy the eggs and embryos. Report any mishap or lost stages, that these may be replaced immediately. Along with the prepared material it will facilitate study to employ enlarged models of the same stages.

1. One-celled Stage.—The greater part of the frog's egg is composed of yolk, which is used for the nutrition of the developing embryo. That portion of the egg in which the yolk is more abundant, the *vegetal hemisphere*, or pole, is marked externally by its white color, while the opposite, in which there is relatively less yolk, the animal pole, is colored with black pigment. Due to the large amount of yolk which it contains the egg of the frog is relatively much larger than that of the starfish but because of pigment granules the nucleus, which lies in the dark pole cannot be seen.

2. Two-celled Stage.—Cleavage begins at the animal pole as yolk retards this process. The depression gradually extends in the form of a groove until it finally surrounds the egg. This groove marks the outer boundary of a cleavage plane which extends through the egg, dividing it into approximately two equal

cells. This first cleavage plane may be spoken of as extending in a vertical or meridional direction.

3. Four-celled Stage.—The next cleavage plane also extends in a meridional direction but is at right angles to the first, resulting in four more or less equal cells.

4. Eight-celled Stage.—The third cleavage plane is said to be equatorial, although appearing nearer the animal pole. It is at right angles to the other two. The result is four smaller cells in the animal hemisphere and four larger ones in the vegetal hemisphere. This inequality in the size of the blastomeres (cells) in the two hemispheres is the result of a relatively small amount of cytoplasm and large amount of inert yolk material in the vegetal hemisphere as compared with the same components of the animal hemisphere.

5. Sixteen-celled Stage.—At this stage in the development of the egg two vertical grooves at right angles to one another appear simultaneously, thus indicating the directions of the next cleavage planes. These grooves first cut the 4 cells of the animal pole into approximately equal halves, forming a 12-celled state, and then continue through the cells of the vegetal hemisphere to complete the 16-celled stage. Either the 12- or the 16-celled state may be found in the material.

6. Crescentic Groove Stage.—From this stage on, the inequality in the size of the cells of the two hemispheres coupled with other tendencies results in irregularities in the number and shape of the cells. These later stages are marked by the appearance of the segmentation cavity within. The *blastula* differs from that of the starfish in the presence of much thicker walls on the vegetal side. The thicker walls of the vegetal side comprise large yolk-laden cells. As a result, the process of gastrulation is very much modified in the frog from that exemplified by the starfish. The large accumulation of yolk at the vegetal side of the blastula prevents the invagination of this region from taking place so that the gastrula is formed partly by a process of ingrowth and partly by the growth of the animal pole over the vegetal pole. The ingrowth and overgrowth take place more on one side of the egg than on the other and are indicated externally by the appearance of a crescentic groove. This groove marks the advancing edge of the down-growing cells of the animal pole. The crescent represents the beginning of the *blastopore* and with its appearance the development passes into the *gastrula* stage.

7. Yolk-plug stage.—The crescentic groove is deepest at the center and thins out toward the edges, which gradually extend around the vegetal pole of the egg. In this way the crescent becomes a circle and as the white-cell area is gradually overgrown a stage is reached when only a small circle of white yolk appears in the midst of the black covering. This is known as the *yolk plug*, socalled since the yolk cells appear as a plug in the blastopore. The white cells which have been enclosed by the dark and other cells which have been overgrown or invaginated now become the *entoderm*.

By the time the yolk-plug stage is reached the formation of the *mesoderm* or middle germ layer is well underway. It develops from the cells around the blastopore which proliferate and push their way in between the ectoderm and entoderm with the result that the embryo comes into the possession of three layers of cells. These are the primary germ layers, furnishing the cells out of which the completed body is formed. The method followed is that which may be expressed as infoldings, outfoldings, and thickenings of the layers and special changes in the component cells.

8. Neural-fold Stage.—This stage is recognized in its early phases by the elongation of the embryo and by the appearance of a fold on each side of a median groove extending lengthwise along the future back of the individual. The edges of these folds, the *neural folds*, later meet and fuse along the median dorsal line, thus forming a longitudinal tube which sinks beneath the ectoderm from which it is separated and forms the central nervous system. The yolk plug has finally disappeared by further overgrowth, leaving only a minute pit to indicate the former position of the blastopore. This pit may be covered over by the neural folds in this region but a short distance behind this point the *anus* begins as an invagination of the ectoderm which later meets and fuses with the archenteron to establish an opening, the anus, between the latter and the exterior.

The beginning of the *mouth* may be seen as a hollow depression of the ectoderm, but it does not communicate with the archenteron until after hatching.

Dorso-caudad of the ventral suckers there develops an elevation in which two vertical grooves appear. These grooves represent the first two gill slits. Later two more grooves appear, one cephalad and one caudad of the first pair. At the time of

hatching all four meet corresponding evaginations of the entoderm of this region thus forming the functional gill slits. The *nostrils* appear as a pair of external depressions or pits a little above the rudiment of the mouth. It is difficult to make out the beginning of the *eyes* which are indicated on each side by an ectodermal thickening, above the nasal pits. Soon after this stage, life in the jelly of the egg mass is abandoned. This marks the end of embryonic existence and the beginning of larval life as a free-swimming organism.

9. Young Larva (Two to Four Days after Hatching).—The features adapting the larva to an aquatic life are easily recognized. Note the vertically flattened tail useful in swimming. The sides of the tail show the zigzag *muscle segments.* Identify the *eyes* on the sides of the head, the ventrally located *mouth,* the two *nasal openings* cephalad of the mouth, and the *external gills* which appear as long, branching tufts extending from the side of the "neck region". The *gill slits* show beneath. This stage in the larval life is of a few days' duration only. The disappearance of the external gills is accompanied by the caudal growth of a flap of skin, the *operculum* (meaning "cover") beginning in front of the first external gill. This cover fuses along its edges with the integument of the head, thus concealing the region of the gill slits along with the surface of the "shoulder region" from which the arms are formed. The edges of the caudally growing opercular flap, throughout its extent, fuses with the surface of the body excepting at one place on the left side where it remains open as the *spiracle.* The external gills are thus covered over and disappear. Internal gills formed within the margins of the gill slits become the functional respiratory organs. With the loss of the external gills and the completion of the tail crest the early larva enters the *tadpole stage.* The true tadpole stage endures until development is completed in which state the larva is said to be mature. During the tadpole stage the legs may be seen growing from the base of the tail. The arms, however, are not to be found upon the exposed surface. If a slit were made through the spiracle and thence cephalad across the opercular flap the relations of the spiracle and gill slits and the location of the arms would be revealed. In the leopard frog larval maturity is reached from 60 to 80 days after the eggs are laid. At this time the larva passes into its third stage, namely, *metamorphosis.* That is, there ensues a relatively short period during

which it loses its equipment for strictly aquatic life (larval characteristics) and assumes the form, structure, and habits of the adult. There can be observed during the metamorphosing period the resorption of the tail, the extension of the mouth cleft, the elevation of the eyes, the perforation of the integument of shoulder region by the arms, and many other transformations. If time permits, study preserved specimens or models of the three larval periods of the frog.

Arrange the material provided for study (*i.e.*, segmenting eggs, embryos, and larvæ) in order ranging from the earliest to the latest stages and demonstrate to the instructor, pointing out not only the stages themselves but also characteristic features of each.

AN INTRODUCTION TO THE STUDY OF INHERITANCE

In preceding studies attention has been called to the behavior of the chromosomes in two kinds of mitosis. In one, somatic mitosis, the chromosomes split equally as regards the quality and quantity of materials which they comprise. Thus, two daughter nuclei are formed exactly like each other and the resulting daughter cells are provided with the normal number of chromosomes. In the other, reduction mitosis, the number of chromosomes in the daughter nuclei through synapsis is reduced by half. Such nuclei become those of the gametes. That is, a mature ovum and a mature spermatazoon each possesses one-half the number of chromosomes normal to the species. A zygote (new individual) formed by the fusion of two such gametes is not only provided with the normal number of chromosomes but also half comes from each of the two parents. While the offspring of a given pair of parents differ from one another in certain details they nevertheless resemble each other more than they resemble the individuals of any other group. This means that there have been transmitted to the offspring the character-potential of the parents which may become expressed and recognized. In other words, the offspring biologically inherit from two parents (biparental inheritance), each of which have inherited from two parents, and so on back. It is, therefore, readily understood that each individual harbors in its chromosomes a great complex of heritable traits which may become expressed according to chance combinations, thus accounting for both similarities and differences which may be encountered among the offspring.

If heredity be interpreted as meaning "organic resemblance based upon descent," no one can question its importance in the study of the rise and perfection of animals. As one of the principle methods of attack upon the problems of heredity is experimental breeding, it has seemed advisable to introduce a study of a simple Mendelian ratio. This study presupposes general familiarity with the subject gained from the textbook, assigned readings, or lectures.

The small fruit fly, *Drosophila melanogaster*, affords excellent material for the study of Mendelian phenomena. It lays its eggs on fermenting fruit the yeast in which forms the food of both the larva and the adult. The complete reproductive cycle from egg to breeding adult is very short, seldom exceeding two weeks. The flies exhibit a number of different eye colors, body colors, and wing characters the inheritance of which may be studied in the laboratory. They are easily grown in bottles containing fermenting bananas.

It will be impossible for the student to carry on the actual breeding during the time allowed for laboratory work but the steps necessary for the preparation of the material are here given that the process and aims may be better understood.

Each group of four students should be supplied with a culture of F_2 flies produced by crosses involving a single Mendelian factor, such as vestigial wing or sepia or scarlet eye, also samples of the P_1 and F_1 ancestors as well as larvæ and pupæ, the object being that of studying the structural peculiarities of larvæ, pupæ, and adults and to observe the inheritance of these factors through two generations and compare the actual count of individuals in the F_2 generation with the expected Mendelian 3:1 ratio. These cultures were produced in the manner set forth in the paragraphs which follow.

Securing the F_1 Generation.—Virgin females for mating were obtained by isolating pupæ in individual half-pint milk bottles containing a banana agar-agar preparation for food. Females cannot be taken from the general stock, as they have almost certainly been fertilized by the males in the culture. In order to secure a pair for the first mating (the P_1 generation) a single pupa was taken from each of the two cultures to be crossed, one from the wild type (red eyed) and one from the sepia-eyed type. Since sex cannot be recognized in the pupal state there are four chances as regards the results, namely, (1) getting a female wild and a male sepia, (2) a male wild and a female sepia, (3) two females, (4) two males. The first two are satisfactory for securing the F_1 generation; if each of the two females resulting from the third possibility is given a male from the original culture these will form two more combinations from which to secure the desired crosses; the two males which may result from the fourth chance is the only combination which is not usable for the purpose of this study. After a pair has been together in the breeding

bottle for about a week they will have mated and eggs will have been deposited resulting in the presence of many larvæ, some of which will have proceeded as far as the pupal stage. As soon as pupæ have formed, the parents *must* be removed so as not to become mixed with their offspring. These F_1 offspring may be collected from such a culture not to exceed ten days after the first flies appear.

Securing the F_2 Generation.—About 15 F_1 adults of each sex are then transferred to breeding bottles in order to insure the production of a large F_2 generation. The expected Mendelian ratio of 3:1 in the F_2 generation resulting from crossing flies in which there is a single Mendelian character involved can be approximated only by having a comparatively large number on which to base a count. As soon as the pupæ have formed, the F_1 adults must be removed so as not to mix with the F_2 flies soon to emerge. Only those flies should be counted which emerge within ten days after the appearance of the first F_2 flies in the culture.

In all holometabolous insects, or those which undergo a complete metamorphosis, four stages in the life cycle are to be expected, namely, the eggs, larval, pupal, and adult. The eggs of the fruit fly will be disregarded because their small size render it difficult to find sufficient numbers for class use. Examine the larva and pupæ of this fly under a dissecting lens and make sketches of each upon the proper plates. Look at a pupa case from which the adult has emerged and note the nature of the exit.

Each table should be supplied with (1) a milk bottle of flies (F_2 generation) living upon a banana culture; (2) a glass vial which will fit over the mouth of the milk bottle and into which the flies are to be driven before etherizing; (3) a black cloth which when wrapped about the milk bottle will facilitate transferring the flies to the glass vial on account of their positive reaction to light; (4) a small bottle of ether and a cotton plug to be used in etherizing; (5) a petri dish into which the etherized flies may be poured for examination, segregation, and counting; (6) a camel's-hair brush for moving the flies.

First, saturate the cotton plug with ether and have it close at hand in order to stop up the mouth of the vial as soon as the flies are transferred to it from the milk bottle. The next step requires much care if all the flies are transferred from one bottle

to the other. The milk bottle *should not* be turned upside down and shaken violently as the banana culture will become loosened and fall with the flies, thus incorporating the flies within its own mass and destroying the results of the experiment. In order to move the flies from one container to another, place a black cloth about the milk bottle and hold the shell vial in the direction of the window or some source of light. The flies being positively phototropic will crawl or fly into the vial where after a few minutes most of them will have assembled. Lift the black cloth from time to time, making sure that none remains in the milk bottle before removing the vial so as to place the ether plug of cotton into its mouth. *A quick transfer is necessary if no flies are to be lost.* Leave the flies in the etherizing vial for about two minutes or until all the flies have dropped to the bottom and then pour them into the petri dish. The work of separating the sepia-eyed flies from the wild flies follows, using the dissecting microscope if necessary, after which the count can be made. If flies become active place a small piece of etherized cotton in the petri dish and cover until movements cease. After the count is made the flies should be returned to the milk bottle and *if not over etherized* may be used again. Avoid loosing flies, therefore, during transfer, and do not leave them in the etherizing bottle too long.

Before returning flies to the milk bottle compare these F_2 flies with the F_1 and the P_1 generations, which will be supplied by the instructors. **Write a description of the male parent used in the P_1 cross** (*i.e.*, a statement of the combination of characters which it possesses). **Do the same for the female used in the P_1 cross. Also a statement of the characters in which the F_1 generation differs from its parents. Then list the number of individuals of each type of F_2 progeny.** How does this compare with the expected ratio?

Two plates are provided upon which a summary of this study may be recorded.

A STUDY OF REPRESENTATIVES OF THE VARIOUS ANIMAL PHYLA

INTRODUCTION

The preceding laboratory periods have been devoted to a study of the organization of a moderately complex animal, the frog. The grosser features of external structure were considered by way of illustrating the importance of the surface of the animal, especially as a means of providing a communicating and buffer systems between the organism and its surroundings. The various internal organs were studied with a view to ascertaining, first, the services which they render and the manner in which it is done and, second, the determination of the interrelationships and interdependence of organs. It became apparent that the body of the animal is an orderly association of components organized according to a plan of differentiation resulting in division of labor among the parts. This organization involves all parts from the cells to the completed body. Each structural component, whatever its rank, contributes a service in the organism as a whole but not independently. Each component in contributing its service is assisted by other components. This requires harmony of action or the coordination and control of the various parts. Such coordination rests largely with the nervous and hormonic forces. For example, respiration cannot be considered as a function of any particular organ. There are involved not only the services of the respiratory organs as such but also the circulation, muscles, the surrounding medium, nervous and hormonic activities, the internal environment, and changes taking place in the protoplasm of the body. Respiration is rather a function of the organism. And so it is with digestion, excretion, etc. Thus it appears that the organism is a complex of interactions taking place in relation with the protoplasm of the component parts of the body and between it and the internal and external environments. When life ceases the components remain in the same state of organization as before death but what is left is a corpse rather than an organism. That is, the *inter-*

actions constitute the organism and as such it is to be regarded as superior to its structural components which it builds for itself.

The laboratory studies throughout the remainder of the course will be concerned with representative types of each of the animal phyla. This is an attempt to decipher through the study of living animals those features of organization which have endowed animals with such advantages as have enabled them to advance beyond a given rank. It is also a means of plotting the probable pathway in the descent of animal groups.

The animals thus studied will be arranged in a series in which the succession is from the lower to the higher.

Review the various grades of groups by means of which animals are classified.

PHYLUM PROTOZOA

Class Sarcodina (*Amœba proteus*).—The amœba is an organism which affords an opportunity for studying living protoplasm in a relatively simple state, since it exists as a small naked mass of this living substance.

Place a few drops of the culture containing amœbæ on a slide, cover with a thin cover slip, and allow it to stand for a few minutes until some of the animals have emerged from the detritus of the preparation. Cut down the light and look for specimens using the *low*-power objective of the compound microscope. When an irregularly shaped, granular, slow-moving object has been located ask the instructor if the desired animal has been found. These animals appear iron-gray in contrast with the yellowish-brown color of the plant tissues in the culture. Select an active specimen in a clear field and study to determine the mode of locomotion.

Observe that the outline of the animal is irregular and variable due to the extension of projections, called *pseudopodia*, which are constantly changing in position, number, size, and form. By flowing into these projections the animal moves with the type of movement known as *amœboid*. The pseudopodia are termed "organs of locomotion," since it is through these that movement from place to place is effected. Notice the outer, thin, clear, firmer layer of protoplasm, called *ectoplasm*, free from granules, and an inner granular, more liquid mass, the *endoplasm*. Do form and locomotion indicate that there are anterior and posterior ends in the animal?

Study the formation of a pseudopodium. The first indication of its appearance is a bulging of the ectoplasm. This is followed by a current of endoplasm into the extended ectoplasm. Because these extensions form only a temporary means of locomotion they have been given the name "pseudopodia." These temporary organs appear capable of extending from any part of the surface.

Make a number (five or six) of one and one-half inch outline drawings of the amœba at intervals of a minute or more, to show successive changes of shape. By arrows indicate the direction in which the protoplasm is flowing in different parts of the animal, at the time the figures are drawn.

Study the structure of a favorable specimen (one whose protoplasm is not too granular) and look for the following features, using the high power.

1. Bear in mind that this is a *unicellular* organism. It is composed of *cytoplasm* as the main mass, within which one or more granular nuclei can often be seen in the living specimen. If the nucleus is not visible in the active specimen study stained preparations. Before considering the structure of an amœba it will be of advantage to have in mind one of the chief differences between this single-celled animal and a single tissue cell or a fertilized egg (zygote) from which all multicellular animals arise. The single-celled amœba is independent of all other cells, while a tissue cell is dependent upon others in the association, and a fertilized egg cannot exist indefinitely without dividing into a number of cells, thus forming an interdependent and interacting association of cells.

2. Search the non-moving part of the endoplasm for a spherical clear spot. At intervals it contracts and disappears to reappear later and does so with more or less rhythm. Because of its nature and behavior it is called the *contractile vacuole.* Observe several pulsations and estimate the frequency. The contractile vacuole is a space so fashioned and functioning as to eliminate liquids from the interior of the cell.

3. The endoplasm also contains particles of ingested food the larger of which are surrounded by a fluid and hence are called *food vacuoles.* Other cell inclusions may be found such as *oil droplets, crystals,* and perhaps foreign particles.

If possible find a specimen which is taking in food and watch the procedure. Observe the method of passing an object with

which it has come in contact. Should a specimen be found in the act of dividing by *simple fission* watch the process closely.

One sees only a relatively slight amount of visible differentiation exhibited within the protoplasm of the amœba yet all of the essential processes of life are in progress as effectively, apparently, as in the frog. The amœba takes food and oxygen, distributes them to various parts of its unicellular body, providing the materials for energy liberation and the building of new protoplasm; it excretes waste, it can move from place to place, respond to stimuli, and reproduce its kind. Physiologically the highest multicellular organism can do no more. Such forms as the amœba, therefore, are of the greatest interest and value to the student of life.

Make an enlarged drawing (four inches in diameter) of an amœba and show those details of structure which you have been able to identify. Indicate the granular appearance by stippling.

Class Infusoria (*Paramœcium caudatum*).—Because of its relatively large size and the ease with which unlimited supplies of specimens may be secured this animal lends itself to the study of the structure and normal activities of a more complex type of protozoan. Members of the class Infusoria possess both permanent form and organs of locomotion. These latter are in the form of *cilia* which are similar in appearance and action to those found upon the surface of the cells lining the roof of the frog's mouth. These permanent locomotor organs of paramecium afford a striking contrast to the transitory pseudopodia of the amœba which are formed "when needed" by a local bulging of the protoplasm of the cell. The permanent form is due to the condensation of the ectoplasm, which necessitates a number of permanent organs some of which are described below. Cilia are responsible for the rapid movements characteristic of this organism.

With a pipette place a drop of the infusion containing paramecia on a slide, cover, and examine under low power. Among the various organisms in the preparation, relatively large, slipper-shaped forms will be noted. These are paramecia. Observe them carefully to determine from observation whether the body possesses permanence of form. Are there differences in the form of the two ends of the body? What is the relation of the ends to the direction of locomotion?

The most striking structural feature to be noted in the swimming paramecium is the depression or broad curved groove which interrupts the even surface of the foremost end. This concavity, the *oral groove,* extends diagonally from the tip of the foremost end to a point beyond the middle of the body, where it terminates in the "mouth." Study the relative widths of this groove and the body at different places, compare the length of the organism with its width, and determine the angle of slant of the oral groove when the oral surface (that in which the mouth is located) is toward the observer.

Make an outline drawing about five inches long of that aspect of the animal in which the oral surface is toward the observer. Only the general form of the body and the oral groove need be shown, but as details of structure will be added later special care should be used in representing proportions.

Various methods have been employed to decrease the rapidity of movement of both animal and its cilia, thereby facilitating the study of details. Before trying any of these, however, look for animals near a piece of scum or other foreign materials which are usually abundant in the cultures. Paramecia usually remain relatively quiet around such material and are studied most satisfactorily when in a normal state. Failing in this ask your instructor for suggestions as to methods of slowing down the activities of these animals.

Compare the degree of differentiation of structures to be found in paramecium, when studied under high power, with that observed in amœba, as suggested in the outlines which follow.

The cytoplasm of paramecium comprises two regions, *ectoplasm* and *endoplasm.* The stratified ectoplasm is rather highly differentiated. The outer layer, or *cuticle,* is a firm membrane to which the animal owes its permanent shape. Study a demonstration preparation of an animal in which the trichocysts have been discharged following stimulation with weak picric acid. Long threads of the discharged contents of the trichocysts can be seen surrounding the animal. How does their length compare with that of the cilia? These structures are supposed to be both offensive and defensive in nature. The innermost or *cortical layer* of the ectoplasm contains the main portion of the trichocysts as well as the two pulsating *contractile vacuoles.* These, aside from eliminating water, possibly aid the animals in maintaining their balance. The two vacuoles appear

one near either end of the animal as circular clear spots into which from seven to ten starlike radiations convey liquids from outlying regions of the protoplasm. These *radiating canals* may be seen at the times the vacuoles contract and are best seen in preparations which are beginning to dry.

The central mass of protoplasm in the animal, the endoplasm, appears devoid of particular structures when examined without special treatment. It is much more fluid than the ectoplasm and appears granular. It generally contains a number of *food vacuoles*, with food in various stages of digestion. The two nuclei, a large *macronucleus* and a small *micronucleus*, are embedded therein. It is usually very difficult to make out either in normal animals. Study a stained slide for an idea of these structures. The macronucleus is a large lobed mass near the center, and the micronucleus is a small spherical body lying in a concavity of the macronucleus. The micronucleus plays an important rôle in reproduction.

The food of this animal is mainly bacteria, decaying organic matter, and minute protozoa. The mouth is located at the base of the oral groove and opens into a funnel-like, S-shaped *pharynx* or *gullet* which leads obliquely toward the hindermost end into the endoplasm. The arrangement of cilia about the oral groove is such that a steady current bearing food particles is directed toward the mouth. If possible, observe an animal feeding. Food particles are wafted down the pharynx by its cilia which are fused into a so-called *undulating membrane*. The particles collect into *gastric vacuoles* at the end of the pharynx. These food vacuoles are then carried through the protoplasm in a more or less definite course during the process of digestion and absorption and the undigested portion is voided through a small permanent *anal spot* in the surface of the body just behind the pharynx. The firm cuticle, giving definiteness of form, has necessitated the presence of oral groove, mouth, pharynx, and anal spot as permanent organs.

Reproduction takes place by *transverse division* (binary fission) of the animal into two daughter individuals. Associated with binary fission are the phenomena of *conjugation* during which the oral surfaces of two animals are connected by a protoplasmic bridge. Across this bridge there is an exchange of micronuclear material. Illustrations of fission and conjugation may be found in the culture, but, if not, demonstration mounts of stained

specimens should be studied. Conjugation may be looked upon as a simple form of syngamy.

To the outline drawing already made, add all the details of structure which have been observed. Such structures as ectoplasm, endoplasm, cilia, contractile vacuoles (showing radiating canals in connection with one), macronucleus, micronucleus, and gastric vacuoles should be indicated. If the trichocyst threads were seen, add only a few to one side of figure to show their relative length. In the lower left- and right-hand corners of the drawing sheet make sketches of binary fission and conjugation.

A STUDY OF MIXED PROTOZOAN CULTURES

A study of mixed protozoan cultures is introduced for the purpose of illustrating the great variety of forms and modes of life of these minute organisms and that of providing an opportunity to gather first-hand information concerning them. A further object in this study is that of furnishing an introduction to the identification (classification) of animals through the use of so-called "tables" or "keys." Such keys make use of the important structural features wherein animals resemble or differ from one another. The statements of characteristic features are so contrasted that the problem becomes one of deciding with which of two contrasting statements a given animal agrees.

Place a drop of the culture in the center of a glass slide. After placing a cover glass upon the drop, study the culture with the low power of the compound microscope. Note differences in form, size, and behavior of the individuals coming within the field of the microscope. Move the slide so as to bring different regions of the culture into the field. Such observations should give one a general idea of the different kinds of protozoans present, their means of locomotion, and noticeable peculiarities.

After a short period the organisms will become less active, enabling one to focus the microscope upon a single individual for a detailed study which will reveal those peculiarities whereby it may be distinguished from other kinds. Look for those characteristics mentioned in the first statement of the key designated by A. The contrasting (opposite) characteristics will be found under AA in the same vertical column. When the characteristics given either under A or AA fit those of the organism proceed to the statement immediately following, and so proceed until a *name* appears at the end of the statement. If the observations are correct this name will be that of the group to which the organism belongs. Reading both of the contrasting statements will aid one in determining which is descriptive of the organism in question.

Assistance in the identification of these organisms may be gained through comparisons made with the accompanying figures.

The matching of organisms with figures is not to be recommended as a general method, but in an introductory study of micro-organisms it facilitates the gaining of familiarity with the different types.

If needed, further explanation in the use of the key will be given by the instructor.

Key for the Identification of Protozoans Most Commonly Found in Fresh-water Cultures

Should forms be found which do not appear to be included in this key, they need not be identified.

A. Locomotor organs not cilia.
  B. Flagella as organs of locomotion. . . . . .Class *Mastigophora*
    C. Solitary (not colonial).
      D. With test formed in plates; one anterior horn and one to three posterior horns; groove encircling body. . . . .*Ceratium*
      DD. Without test.
        E. Colorless; *i.e.*, without green, yellow, or brown chromatophores.
          F. Body truncate or concave at anterior end, slightly flattened; two flagella. . . . .*Chilomonas*
          FF. Body not truncate at anterior end.
            G. Body elongate, wider at posterior end; two flagella, one long and heavy, one short and fine. . . . .*Astasia*
            GG. Body oval, flattened, not wider at posterior end, and very flexible, gullet present with rodlike organ back of mouth; one flagellum. . . . .*Peranema*
        EE. With green chromatophores.
          F. Body not spindle shaped.
            G. Body spherical, or eliptical in form with one large cup-shaped chromatophore and stigma (eyespot); two flagella. . . . .*Chlamydomonas*
            GG. Body round or pear shaped, not symmetrical, with caudal process; one flagellum. . . . .*Phacus*
          FF. Body spindle shaped; gullet present; single flagellum; stigma present. . . . .*Euglena*
    CC. Individuals associated in colonies.
      D. With yellowish brown chromatophores.
        E. Colony spheroidal.
          F. Individuals embedded on surface of a gelatinous mass; two yellow chromatophores. . . . .*Uroglena*
          FF. Individuals not embedded in gelatinous mass and loosely joined; two flagella. . . . .*Synura*
        EE. Colony arboroid; each individual resting in a cellulose cup; one long, one short flagellum; the cup of each individual attached within the cup preceding it. . . . .*Dinobryon*

DD. With green chromatophores.
E. Colony flat (cells in one plane) of 4 to 16 individuals; flagella directed from one surface . . . . *Gonium*
EE. Colony spheroidal.
F. Individuals equal in size.
G. Individuals crowded, inner ends pointed and reaching center of mass; colony enclosed within definite membrane; two long flagella . . . . *Pandorina*
GG. Individuals scattered in jelly mass (not crowded); two flagella. . . . . *Eudorina*
FF. Individuals not all of same size (smaller ones vegetative, larger ones germinal).
G. No protoplasmic connections between cells; larger germ cells at posterior pole of colony (*i.e.*, poles of colony differentiated). . . . . *Pleodorina*
GG. Protoplasmic connections between cells distinct; poles of colony not differentiated. . . . . *Volvox*
BB. Locomotor organs in form of pseudopodia which may be changeable, blunt, fine, or raylike and rigid. . . . . Class *Sarcodina*
C. Pseudopodia fine and raylike; no outer envelope or shell, body rounded.
D. Ectoplasm not distinctly differentiated, axial filaments of pseudopodia extending to nucleus. . . . . *Actinophrys*
DD. Ectoplasm distinct, axial filaments extending to ectoplasm *Actinosphaerium*
CC. Pseudopodia not raylike but changeable and flowing; body form irregular.
D. Body naked (without test). . . . . *Amœba*
DD. Body with test.
E. Test chitinous, smooth, hemispherical in form, provided on its flat surface with circular opening through which pseudopodia extend. . . . . *Arcella*
EE. Test covered with minute foreign bodies and therefore appears uneven. . . . . *Difflugia*
AA. Cilia present as locomotor organs. . . . . Class *Infusoria*
B. Mouth surrounded by a spiral zone of large cilia.
C. Free-living, membranelle winding to left.
D. Entire surface of cell covered with cilia.
E. Adoral zone (fringe of fused cilia near mouth) nearly at right angles to body axis; cell trumpet shaped; free swimming (or may become attached temporarily but without special stalk). . . . . *Stentor*
EE. Adoral zone paralleling body axis.
F. Adoral zone funnel shaped, sunken in the body; cell oblique in front. . . . . *Bursaria*
FF. Adoral zone narrow, elongate, reaching middle of body, which is elongate in appearance. . . . . *Spirostomum*
DD. Ventral surface with cirri (long cilia fused together to form bristle-like appendages, which are sometimes used as legs); dorsal surface with only fine cilia or none.

E. With both cilia and cirri.
F. Body not very flexible.
G. Three caudal or posterior cirri. . . . . *Stylonychia*
GG. No caudal cirri. . . . . *Histrio*
FF. Body quite flexible; caudal cirri undeveloped. . . . *Oxytricha*
EE. With cirri only.
F. With four caudal cirri. . . . . *Euplotes*
FF. With seven to nine caudal cirri. . . . . *Uronychia*
CC. Sessile, enlarged oral cilia winding to right, usually forming an almost complete circle.
D. Solitary, attached by a retractile stalk; body bell shaped; circle of strong cilia about elevated disc of free (broad) end. *Vorticella*
DD. Individuals forming arboroid colony; bodies bell shaped.
E. Stalks retractile.
F. Individuals contracting independently. . . . . *Carchesium*
FF. Individuals all contracting together. . . . . *Zoothamium*
EE. Stalks not retractile. . . . . *Epistylis*
BB. Mouth not surrounded by a spiral zone of large cilia.
C. With a sculptured shell about a barrel-shaped body. . . . . . . *Coleps*
CC. Without such a shell.
D. Cilia not uniform over body.
E. Prominent cilia confined to one or two bands.
F. With two circles or crowns of long cilia, one near the anterior end, the other in the region of the equator of the ovoid body; nipple-like elevation bearing mouth and arising from the center of a flattened anterior end *Didinium*
FF. Two zones of strong cilia about body; cell somewhat cylindrical, with a constriction slightly behind the middle; a prominent tuft of fused cilia at the posterior end *Urocentrum*
EE. Cilia only on ventral surface; cirri not present.
F. With a long neck; body oval, ending in a short tail. *Lionotus*
FF. With the anterior end hooklike; body inflexible, ventral surface flattened and showing ciliated ribs; mouth on the left anterior edge, at the bottom of a slitlike peristone. . . . . *Loxodes*
DD. Cilia uniform over body.
E. Mouth terminal or nearly so; no proboscis.
F. With no evident gullet; body elongate, anterior end narrowed and flattened into a neck which is obliquely truncate. . . . . *Spathidium*
FF. With more or less evident gullet.
G. Form flask shaped; with a short or long, highly contractile neck, and a rounded posterior end. . *Lacrymaria*
GG. Form spherical to ovate; without a neck; cytopharynx long. . . . . *Prorodon*
EE. Mouth somewhat posterior to anterior end; sometimes with a proboscis.
F. With proboscis in front of mouth.

G. Body elongate; mouth at base of a long, very flexible proboscis . . . . *Dileptus*

GG. Body flattened, leaflike; proboscis only slightly developed . . . . *Loxophyllum*

FF. Without proboscis.

G. Mouth anterior to middle.

H. Form ellipsoidal; mouth not far from anterior end; genus includes some of the largest holotrichs *Frontonia*

HH. Body somewhat kidney shaped; mouth about one-third of the distance back of the anterior end; species small as compared with those of Frontonia . . *Colpoda*

GG. Mouth near middle; gullet short; peristome oblique *Paramecium*

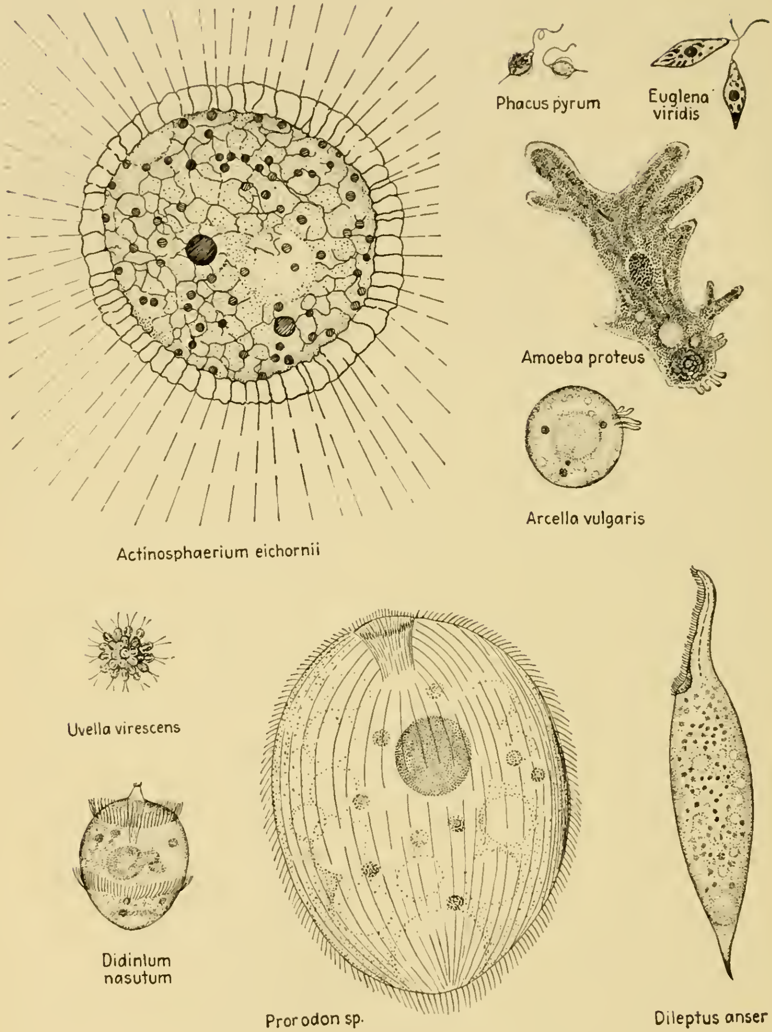
Phacus pyrum
Euglena viridis
Amoeba proteus
Arcella vulgaris
Actinosphaerium eichornii
Uvella virescens
Didinium nasutum
Prorodon sp.
Dileptus anser

FIG. 3.

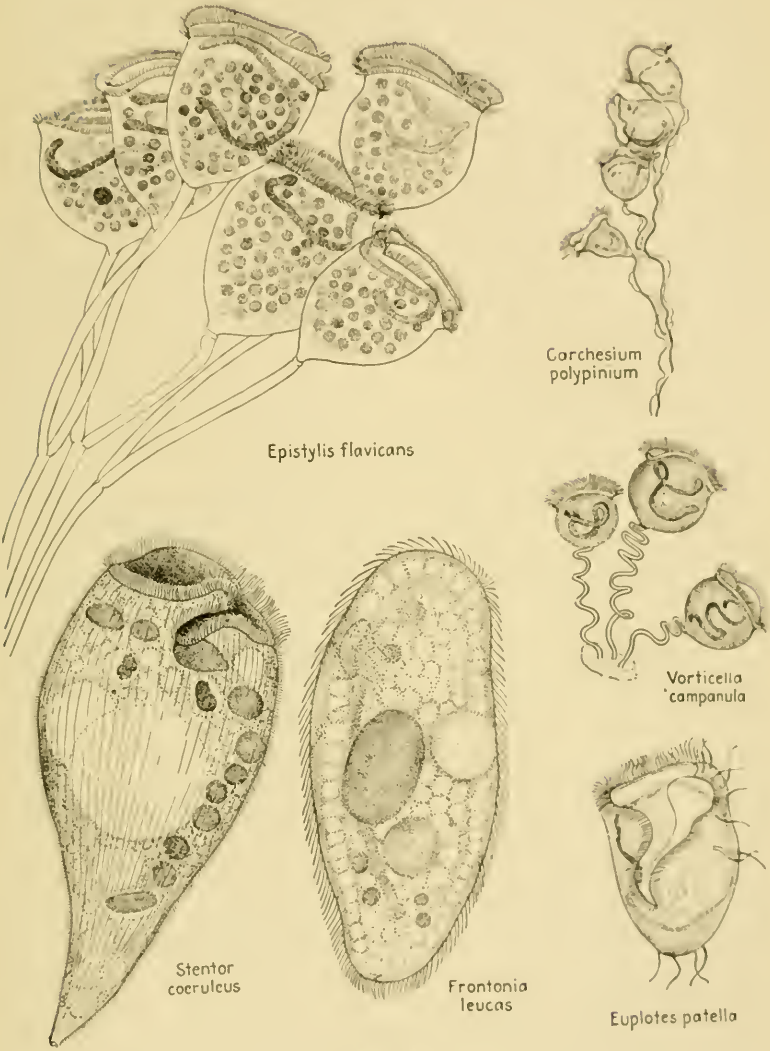
Carchesium
polypinium
Epistylis flavicans
Vorticella
campanula
Stentor
coeruleus
Frontonia
leucas
Euplotes patella

FIG. 4.

PHYLUM CŒLENTERATA

In the phylum Cœlenterata there is laid down the fundamentals of body organization which are adhered to in all higher types of animals. In contrast to the sponges the representatives of this phylum fall in the direct line of animal descent. Only two cell layers are found in the body wall. The cells of both layers are differentiated in relation to definite functions. All of the cœlenterates are aquatic, mostly marine, and chiefly sessile in the adult state.

Class Hydrozoa (*Hydra*).—Specimens of Hydra may usually be found in quiet ponds or streams where they are attached by one end to aquatic vegetation.

Examine a living individual in a watch glass with a hand lens, or with the low power of the compound microscope.

Jar the watch glass and note any response to such a mechanical stimulus. With the point of a needle very gently touch one of the tentacles of an extended animal. Is there any evidence that the stimulus is transmitted from one tentacle to another or to any other part of the body? Compare the form of the body when extended and contracted. If a hydra should be found moving along the object to which it is attached note the type of locomotion employed. Reflecting upon the hydra's behavior, what deductions might be made with regard to some details of organization?

General Structure.—The body of the animal in a moderately extended state is tubelike in form increasing slightly in diameter toward the free end so that under certain conditions it is vaselike. At the fixed end the body expands into a *basal disc* for attachment. A circle of slender *tentacles* arises from the free end. These may vary in number. In the arrangement of bodily parts Hydra is radially symmetrical, that is, similar parts are repeated about an axis as a center.

The region encircled by the bases of the tentacles projects as a conical elevation termed the *hypostome*. At its apex is located the *mouth*, which leads into the digestive cavity. The mouth is usually closed and difficult to distinguish.

Observe the irregular appearance of the surface of the tentacles and body wall. These small elevations indicate the location of batteries of stinging cells which are found on all parts of the body except the basal disc but are most numerous on the tentacles. The high power of the microscope reveals each battery as a compact group of specialized cells called *cnidoblasts* within each of which is the stinging mechanism or *nematocyst* in the form of a pear-shaped capsule. Prepared slides should be provided for this observation.

Determine the extent of the *digestive* or *gastrovascular cavity.* By focusing carefully on such a partially transparent organism some of the details of internal structure can be determined by bringing into focus different levels of the body. By thus manipulating the microscope the two body layers may be recognized. The hollow central cavity of the animal appears lighter than the denser edges. Ingested food is forced down into the digestive cavity where the greater part is apparently engulfed by pseudopodia of the cells lining the cavity and digested within these cells in the the protozoan manner. Although digestion is mainly *intracellular* there is also a limited amount of *extracellular* digestion in Hydra similar to that obtaining in the frog and higher animals. Certain cells among those lining the digestive cavity secrete juices containing enzymes which are discharged upon their surfaces where small amounts of food materials are split into simpler compounds. Undigested food is ejected through the mouth.

Both agamic and gamic reproduction occur in Hydra. The common agamic mode is that of budding, according to which a new individual is formed as a bud growing from the side walls of the parent. The buds may be found in varying stages of development. When mature the new individuals separate from the parent, leading an independent though sessile life. *Transverse* and *longitudinal fission* of the whole animal have been reported.

In its gamic reproduction Hydra is *hermaphroditic* and without permanent gonads. During the period of gamic reproduction the gonads appear as elevations of the surface. The *spermaries* form conical elevations in the ectoderm somewhat nearer the tentacles than the base of the animal, while the ovaries form low, broad elevations near the base. Both sperm and egg mother cells arise from interstitial cells and are borne by the same

individual. Although hermaphroditic it is rather exceptional to find specimens bearing both ovaries and spermaries at the same time.

Make an outline drawing, at least five inches in length, of an expanded Hydra as seen from the side. To the same scale draw the animal in a contracted state.

Details of Cellular Structure.—Examine a stained cross-section of the animal using the low power for the preliminary study. Hydra clearly presents the fundamental plan of structure in the cœlenterates. Observe that the cells of the body wall are arranged in two definite layers, an outer *ectoderm* and an inner *entoderm*, the latter forming the lining of the *gastrovascular cavity*. These two layers are sharply marked off from each other by a very thin non-cellular layer of supporting substance, the *mesoglea*.

Study the cross-sections under the high power. The *ectoderm* is composed of three principal kinds of cells. By far the commonest type is the *epithelio-muscular* cell to be distinguished by its large nucleus and deeply staining nucleolus. The bases of these are expanded and contain long *contractile fibers* which form a thin longitudinal muscular layer accounting for the remarkable capacity for contraction which Hydra possesses. These contractile bases cannot be made out in thin cross-sections.

A second type of ectodermal cell is called the *interstitial cell.* These are small, spherical, somewhat granular cells occurring in groups between the epithelio-muscular cells. They appear to be undifferentiated cells held in reserve to take up any one of several functions when necessary. It may be that they give rise to *cnidoblasts* more frequently than to other kinds of cells.

The cnidoblasts are the cells enclosing capsules or *nematocysts* containing the stinging threads. Each cnidoblast contains a nucleus and gives rise to a spinelike projection, the *cnidocil*, at its outer end. Nematocysts show an affinity for eosin and therefore appear stained a pinkish color when treated with this dye. Threads shot out from these cells penetrate the body of small animals and inject into them a fluid which has a paralyzing effect.

At least two types of cells can be distinguished in the entoderm. The more common type is the large, elongate, vacuolated digestive cells. They are dumb-bell-like in shape, the larger end extending into the gastrovascular cavity. Their nuclei and

nucleoli resemble those of the epithelio-muscular cells. At their free ends are two flagella which are not visible in ordinary preparations. The basal ends of these cells are provided with extensions containing contractile fibers.

Between the digestive cells another type of cell may often be seen. These appear as triangular cells with the broader end exposed to the digestive cavity. They are known as *gland cells,* since they secrete the enzymes used in the small amount of extracellular digestion.

The non-cellular *mesoglea* secreted by cells of the ectoderm and entoderm is composed of a gelatinous non-cellular material.

If possible the details of cellular structure should be reviewed by a study of mascerated, teased, and stained cells of the body walls. When such preparations are successful one should be able not only to recognize the cell components of the body walls but also to determine the form and general relations of the cells. Sensory and nerve cells are difficult to find and study in transections and are therefore not included in these outlines.

In a four-inch circle representing a diagram of a cross-section of Hydra show the relative width of ectoderm, mesoglea, entoderm, and gastrovascular cavity. In a small sector of this diagram show in detail the structure of the various types of cells composing the two layers of the body wall.

Class Hydrozoa (*Obelia*).—In addition to Hydra and a few other fresh-water representatives, the class Hydrozoa includes a large number of marine species of diverse form and appearance. These are, however, all reducible to the fundamental plan of structure typified by Hydra. Great numbers of these species are colonial in habit. As an example of a colonial hydroid, Obelia is chosen for study. It is a common marine form found on docks, submerged or floating wood, stones, seaweeds, etc. Obelia commonly occurs in shallow water just below the ebb-tide level. It may be found at greater depths.

In Obelia the striking feature is the division of labor which obtains among the *several kinds of individuals* comprising the colony, each of which is specially modified in connection with the performance of definite functions for the colony as a whole. There are three kinds of polyps (*i.e.*, three kinds of individuals or "Obelia persons"): (1) the *nutritive polyp,* whose service to the colony is food getting; (2) the *reproductive polyp,* whose serv-

ice is the production of medusæ which arise as buds; and (3) the *medusæ,* whose function is gamic reproduction and the dissemination of the species. A colony consisting of three or more different kinds of specialized individuals is said to be *polymorphic.*

The Structure of a Colony.—Certain general features of the colony must be clearly in mind before a detailed study of any part is begun. Under the dissecting microscope examine a prepared specimen of a single stem of an Obelia colony. Note that the main stem is zigzag in form although sometimes in preserved material this is not pronounced. A demonstration preparation of a stem exhibiting its natural state should also be examined. The attached end of the stem, in root fashion, creeps along the surface to which the colony is attached. This portion of a stem may be called the "foot."

The main stem gives off alternating side branches each of which terminates in an individual of the colony. At the extremities of these side branches observe the *nutritive polyps,* characterized by the presence of numerous tentacles. The polyp nearest the foot is termed the *founder polyp,* since it founded the colony.

Less numerous are the *reproductive polyps,* which are usually borne in the angles where the stems of the nutritive individuals arise from the main stem. They may be distinguished from undeveloped feeding polyps by their position on the stem as well as by their swollen appearance.

All the soft parts of the colony are protected by a translucent sheath, the *perisarc,* which becomes expanded and variously modified in the regions of the nutritive and reproductive polyps.

Make a six-inch diagram showing the growth habit of an Obelia colony. Use schematic figures to represent the several kinds of individuals. Label structures described in the preceding paragraphs.

Details of Structures.—Under the low (or, if necessary, high) power of the compound microscope study in detail a portion of the stem and branches containing all types of polyps.

The living tissues of the individuals and stems are called the *cœnosarc,* the reference being to the living flesh in contrast to the perisarc which is composed of inert materials secreted by the cells of the cœnosarc.

The cœnosarc of the main stem will be readily recognized by its granular nature and deeper staining qualities. Note that

the cœnosarc (both stem and body) of each Obelia individual in a colony is continuous with that of the main stem and therefore with every other individual in the colony; also that there is a cavity within the living stems which is connected with the digestive cavity of each individual. Of what advantage is this condition to the animal? Some digestion takes place in the cavity of the stems as well as in the gastrovascular cavity of the feeding polyps but the predominating type of digestion in Obelia, as in Hydra, is intracellular.

The perisarc, throughout, stains more lightly than the cœnosarc and is of a homogeneous, nature. Is it uniform in thickness on both sides of the stem? Is this thickening the same in successive segments? Note the nature of the perisarc surrounding the stems leading to the polyps.

A nutritive individual of an Obelia colony is similar in fundamental respects to a Hydra; the body wall consists of two cellular layers, the ectoderm and the entoderm, and the thin, noncellular mesoglea. *Hypostome, mouth*, and *tentacles* are in the same relative positions and are readily recognized. Compare the tentacles of Hydra and Obelia and determine the most fundamental differences. The perisarc, surrounding the nutritive polyp, is expanded into a goblet-shaped structure, which, since it harbors a polyp, is called a *hydrotheca* (meaning hydra case). Look for variations in the thickness of the hydrotheca. Note the centrally extending ledge at the base of the hydrotheca supporting the body of the polyp.

The reproductive polyps are elongate in form, ending in a broad, flat disk with neither mouth nor tentacles. The walls of these individuals produce by budding, the third kind of individuals in the colony, the *medusae buds*. Observe carefully the structure of one of these. The perisarc is vase-shaped around the reproductive polyps and is known as the *gonotheca*.

The medusæ buds, when mature, separate from the core of the reproductive polyp or blastostyle, pass out through an opening in the end of the gonotheca, and lead a free life. They are to be regarded, nevertheless, as sister individuals to the feeding and reproductive polyps, which remain attached to the colony.

Upon the plate of Obelia label details to which attention has been directed in the preceding paragraphs.

The Study of a Medusa. As stated above, the medusæ buds when fully grown break away from the blastostyle and lead a

free life. Gamic reproduction prevails in this type of individual and the sexes are distinct.

The medusæ of Obelia are small. The medusa of another hydrozoan, *Gonionemus murbachii,* has been chosen for the study of a typical medusa because of its fair size. It is found in considerable numbers through the summer in the vicinity of Woods Hole, Mass.

Structure.—Study a specimen in a watch glass with a dissecting microscope. Use great care in turning the animal over for different views. Never use forceps. A camel's-hair brush serves the purpose much better.

Notice the *umbrella-shaped* body with tentacles hanging free from the margin and the *hypostome* hanging down within the space formed by the concave surface of the umbrella. The hypostome is the same structure already observed in Hydra and Obelia. The convex surface of the umbrella is called the *exumbrella* (or aboral surface), while the concave portion is termed the *subumbrella* (or oral surface).

A circular muscular shelf, the *velum,* extends inward from the edge of the umbrella. By gentle contractions of the umbrella (or bell) and of the velum, water is driven out of the subumbrella cavity, thus effecting locomotion.

To understand the relation of parts comprising the food-distributing system place the animal with aboral side uppermost. Note the quadrangular area visible through the transparent tissues; this area marks the location of a relatively large saclike structure at the base of the manubrium, the *stomach,* from the angles of which four delicate, equidistantly spaced *radial canals* lead to the edge of the umbrella where they open into the *circumferential canal* following a circular course about the margin of the bell. These canals serve as a food-distributing system and together with the stomach represent, as far as spaces are concerned, the gastrovascular cavity of the nutritive polyps.

On the subumbrella side of the radial canals note the corrugated reproductive organs or *gonads* which may be either spermaries or ovaries.

Observe the mode of distribution of nematocysts on the tentacles and compare in this respect with tentacles of Hydra and Obelia. An abrupt bend near the distal end of the tentacles of Gonionemus indicates the location of a cuplike *adhesive*

disc by means of which the animal can attach itself temporarily to vegetation or other objects in the water.

Turn a specimen with the velum side toward the observer and study the edge of the body for the two kinds of sense organs. The low objective may be necessary to render these distinguishable. At the base of each tentacle and communicating with the circumferential canal is a noticeable round body which is made up of strongly pigmented cells. These are considered as *light-perceiving organs.* Other smaller transparent outgrowths between the bases of the tentacles are the *statocysts* which serve as balancing (static) organs. The jelly-like consistency of the medusæ is due to the enormous development of the mesoglea.

Upon the plate of Gonionemus label all the parts to which attention is called in the outlines.

PHYLUM PLATYHELMINTHES

There is a double purpose in the study of this phylum. One is found in the parasitic life which great numbers of these animals lead and the resulting maladies produced in higher animals which serve as hosts. The other purpose is found in their body organization which serves as an index to their relative success as organisms and their elevation to a higher rank than that attained by the forms previously studied. Among other advances there may be noted: (*a*) the mode of progression, being that of an elongate animal moving with the same end of the body foremost, (*b*) the regional differentiation of the body as a whole which accompanies this type of locomotion, namely, a true anterior and posterior end, bilateral symmetry (with right and left sides), dorsal and ventral surfaces, and (*c*) triploblastic body walls which have provided the structural means for these advances in organization.

Class Turbellaria. *Planaria or Procotyla (Dendrocœlum).*—Either of these genera of flatworms is found commonly in fresh-water ponds throughout the United States. The species of Procotyla are white and somewhat translucent, a condition which greatly facilitates the study of the digestive and other internal organs. These free-living (*i.e.*, non-parasitic) flatworms or turbellarians are found under stones, leaves, or other submerged objects.

Behavior and External Structure.—Make observations upon a living specimen in a watch glass, using a hand lens or dissecting microscope. Study the behavior, form, and surface structure of the animal, making use of the following as a guide: (1) form of body, making specific references to the relative length of the three axes (longitudinal, vertical, transverse); (2) relation of body to the substratum (surface over which the animal crawls); (3) relations of the ends of the body to direction of locomotion; (4) movements which involve muscular activity and the normal *gliding form* of locomotion due to the action of cilia covering the body; (5) muscular movements as a means of *crawling*, determining the nature and progress of the waves of contraction; (6)

comparison of muscular movements of the flatworm and those of Hydra; (7) observations and description of the behavior of the tentacles and the anterior end of the animal during locomotion, with conclusions; (8) determination of results of stimulating various parts of the animal with a camel's-hair brush, especially regional difference in sensitivity.

At the conclusion of the laboratory period hand in a written report embodying the results of observations upon the structure and behavior of the flatworm.

Make a five-inch drawing of the animal from the dorsal side. Show and label eyespots, tentacles, and any other structures observed.

In a well-fed animal of the white species the digestive canal may be seen clearly outlined in brown. If such an animal is being studied note the oval clear region, the *pharyngeal chamber* in the center of the body, in which is located an elongate muscular tube, the *pharynx*. The pharynx can be extended as a proboscis through the *mouth opening* located on the caudal part of the midventral side. At its cephalic end the pharynx opens into an *intestine* comprising three main trunks, a *median branch* extending to the cephalic region, and *two lateral branches*, one extending caudad on either side of the pharyngeal chamber toward the posterior end of the body. Each of these branches further gives off a large number of irregular lateral extensions.

In all probability, the majority of the worms available for study will be of the dark species (Planaria). Often these may be induced to eat blood or liver if such materials be added to the water in the watch crystal. If a dark-colored worm is being studied, try such a feeding experiment. When the animal begins to eat, a better idea of the structure of the pharynx or proboscis may be gained upon close observation. A little patience while inducing an animal to feed is often rewarded. The general shape of the gastrovascular system of Planaria is similar to that of Procotyla described above. Study a specimen of Planaria prepared to show the digestive system. Locate the three main divisions and determine the ramifications of each.

Represent the digestive system in the drawing already made.

Flatworms have well-differentiated *nervous*, *excretory*, and *reproductive systems*, but these are so obscure that their study is impossible in a living animal.

Regeneration.—Flatworms show remarkable powers of regenerating lost parts. In each section the instructor will cut a specimen in two while it is gliding across the bottom of a watch glass and place the two halves in a bottle of water to examine later. Each student should make a rough sketch of this worm showing the approximate location of the cut in order to aid his memory when examining the specimen later for evidences of regeneration.

PHYLUM ANNELIDA

The phylum Annelida comprises the so-called "true worms" in contrast to the flat worms. Both phyla are important in tracing advances in organization.

In the flat-worms bilateral symmetry and progression with the same end foremost are associated with noticeable though moderate regional differentiation, as for example, an anterior end which may be regarded as an incipient head. In the annelids the anterior end has become further differentiated. Aside from this advance in their perfection the annelids are organized upon the structural plan of *metamerism* accompanied by the presence of a true body cavity or *cœlom* which is not to be regarded as the least of important annelid innovations.

Class Chætopoda (*Nereis, a sandworm*).—Nereis may be taken as a typical annelid. It lives in burrows in the coarse sand and mud of the seashore at tide level.

External Structure.—Study an animal in a wax-bottomed dissecting pan. Make observations upon the surface structure with a view to determining the kind of symmetry. The most striking structural character is the ringed appearance of the body. Each of these rings is a unit in the organization plan of the body and is called a *metamere* (or *somite* or *segment*). Each metamere bears a pair of very thin lateral prolongations of the body wall, known as *parapodia*, whose double function will be considered later.

Head.—Two segments in the anterior region are so modified as to be able to test the nature of the surroundings as the body advances. Other functions in these segments are lost to a great extent. The first somite, or *prostomium* (meaning "in front of the mouth"), is a dorsal rectangular projection which does not extend to the ventral side. The second, or *peristomium*, is a complete ringlike segment bearing the *mouth*.

The sense organs of the head have reached a relatively high state of perfection. Projecting from the middle of the cephalic edge of the prostomium there are two short *prostomial tentacles*. On either side of these is a thick, jointed *palp* whose service is

probably that of providing a sense subserving as taste. On the dorsal surface of the prostomium are four indistinct grey spots, each marking the location of a *light-perceiving organ.* The peristomium bears four pairs of lateral *peristomial tentacles* or *cirri.*

Make a four-inch drawing of the dorso-lateral aspect of the left side of the head and first segment of the body. Label the parts described above.

Body and Tail Metameres.—The large number of segments caudad of those already mentioned as constituting the head may be regarded as the *body* metameres, excepting the last which is called the *tail* segment. Note the similarity between the body segments. Internally, parts will be found repeated in successive segments.

The posterior or tail segment differs from the others in the modification of the parapodia into a pair of tactile processes or *cirri.* In this segment the *anus* is located.

Parapodium.—Compare the parapodia at different parts of the body for similarities or differences. Study a parapodium which has been removed and mounted in balsam, or cut off one with the scissors, mount in water, and cover with a glass slip. Employ a dissecting lens or the low power of the compound microscope. Compare the parapodium studied in this way with those still attached to the animal and determine which is its dorsal and which its ventral edge.

These lateral outgrowths of the body wall serve both as *respiratory* and *locomotor* organs. A parapodium can be divided into two distinct portions, a dorsal called the *notopodium* and a ventral called the *neuropodium,* each of which is stiffened by an internal chitinous supporting rod, called the *aciculum* or *bristle.* Find the two acicula. The large dorsal lobe of the notopodium is a respiratory organ, that is, a *gill,* containing a network of blood vessels which can easily be seen in life. Attached to its dorsal edge is a slender vibratile sense organ, the *dorsal cirrus.* The ventral portion of the notopodium comprises two lobes the smaller of which bears fine bristles or *setæ.* The neuropodium likewise comprises two lobes, the notched one of which is setæ bearing. Beneath these lobes on the ventral margin of the neuropodium is a *ventral cirrus.*

Make a three-inch outline drawing of a parapodium, labeling the structures described above.

A STUDY OF THE EARTHWORM

The above study of Nereis presented information concerning what may be regarded as the typical external structure of marine worms. There is another great group of worms typified by the earthworm. The worms of this group are terrestrial, though some are aquatic and a few are equally at home in either kind of environment. These animals feed mostly at night by crawling entirely or partially from their burrows to the surface of the ground. Their subterranean habits are associated with the absence of certain structures which are possessed by the marine worms.

The structural features to which attention is called in the following paragraphs apply to the large species, *Lumbricus terrestris*. If other species are studied, allowance must be made for variations in structural details such as number of metameres, the location of apertures, and the relations of internal organs to segments, etc.

External Characters.—Study a preserved specimen in a dissecting pan containing a little water. For the finest structures employ either a dissecting microscope or the lowest-power objective of a compound microscope. Distinguish anterior and posterior ends, dorsal and ventral surfaces. Is the "head" as well differentiated as in the case of Nereis? The head of Nereis is to be regarded as more nearly a typical annelid head, while that of the earthworm is simplified or better fitted to the needs of subterranean life. Do any obvious vestiges of the parapodia persist in the earthworm?

As in Nereis, the mouth is surrounded by the first complete segment or *peristomium* and is overarched by a small lobe, the *prostomium*. Caudad of the peristomium the similarity of the body segments is quite noticeable, each being readily determined externally by the grooves extending around the body. Determine the total number of metameres exclusive of the prostomium. In the cephalic third of the body of mature worms certain segments are swollen forming about the body a thick band or girdle called the *clitellum*. This is a glandular region which secretes the cocoon in which the eggs of the earthworms are deposited. Counting from the anterior end and omitting the prostomium determine the metameres occupied by the clitellum.

Pass the fingers in both directions along the ventral part of the sides of the body. The sensation produced is due to rows

of bristles or *setæ* which aid the worm in crawling. These structures render the removal of these animals from their burrows very difficult. Determine the relations of the setæ to each other, to the metameres, and to the symmetry of the body.

The body of the earthworm is covered by a delicate transparent *cuticle* secreted by the cells which lie immediately below it.

Are there any eyespots to be found? While unable to hear, these animals are very sensitive to vibrations conveyed to the body through the ground and also to various forms of chemical stimuli and light.

In addition to the *mouth* at the anterior end and the *anus* in the last segment numerous other external openings are found on the ventral side of the body. The *nephridiopores*, openings of the excretory organs, should be located previous to dissection of the specimen. With the exception of a few somites in the cephalic end and the last somite, a pair of these openings occurs in every segment. They are usually situated somewhat diagonally in front of the outermost setæ of the ventral (inner) double row. If these openings cannot be located examine a specimen under the demonstration microscope.

Carefully examine the ventral surfaces of segments 14 and 15 for the genital apertures which are larger than those of the nephridial tubes and differently located. The openings of the vasa deferentia are upon rounded elevations one on each side of the ventral surface of segment 15. The oviducts open in a similar location upon segment 14 but are not borne upon elevations.

The food of the earthworm consists of organic matter contained in the soil which is drawn into the mouth by the bulblike pharynx. This is controlled by small muscles radiating from its walls to the body wall.

In order to study the digestive system the animal should be dissected under water. By means of pins through the two ends fasten a preserved specimen in water, dorsal side up. *Because the alimentary canal lies close to the dorsal body wall great care should be used in performing the following dissection.* With forceps hold up the body wall at a point about one inch behind the clitellum and with the points of the scissors make an incision a little to the left of the median line. Carefully continue this cut forward to the prostomium. In order to make it possible to pin out the body wall on each side and thus expose the internal

organs it will be necessary to snip with the scissors or cut with a scalpel the septa which hold the body wall close to the alimentary canal. Separate the edges of the cut, spread open the walls of the body, and fasten by means of pins inserted diagonally near the periphery in segments V, X, and XV.

At the point where the first incision was made note that the body wall is separated from the intestine by a distinct space, the *cœlom*. Do you find any indications of metamerism internally? What is the relation of the *septa* to the external rings? Note the glistening, iridescent lining of the body wall. What is the relation of this lining to the cœlom?

The large cream-colored structures somewhat obscuring the alimentary canal in segments 10, 11, and 12 are the three pairs of *seminal vesicles* which are parts of the reproductive system. Laterad of the anterior pair of vesicles in segments 9 and 10 are small white spherical objects, the *seminal receptacles* (spermathecæ), which are also parts of the reproductive system.

Along the dorsal surface of the intestine can be seen a brown or yellow streak which marks the location of the longitudinal ridge, the *typhlosole*, which encroaches upon the enteric cavity. Its color is due to the yellow *chlorogogen* cells of which it is mainly composed. The typhlosole can be more readily distinguished later when a section of the intestine is removed. Note also the *dorsal blood vessel* visible along this streak. Arising from it are transverse vessels in each segment, best seen in the region of the intestine. One pair of these in each of the seventh to the eleventh segments, inclusive, on either side of the œsophagus, is specialized as the *hearts*, which due to their pulsations assist in maintaining a blood current.

The Digestive Tract.—Identify the following regions in the digestive tract: the *mouth* or *buccal cavity* in the first three segments; the longer, thick-walled *pharynx* extending through about four segments and attached to the body wall by radiating muscle fibers; the narrower *œsophagus* (partly concealed by the five pairs of hearts and by reproductive structures) extending to segment 13 or 14; the large, thin-walled *crop* or storage reservoir for food, extending through three or four segments; and the white, thick-walled *gizzard* in which the food is ground into small particles. From the gizzard the *intestine* stretches to the anus as a long unspecialized tube. It is in the intestine that digestion is completed and absorption takes place.

On the plate of the anterior end of the earthworm designate the parts of the head; then label the parts of the blood system and digestive tract. On the small drawing in the lower right-hand corner label the space representing cœlom and the location of the setæ. Write the names on the left of this diagram.

The Excretory Organs.—The excretory system of the earthworm consists of a series of paired coiled tubes, the *nephridia*, one pair of which is present in every segment except the first three and the last. Remove a portion of the alimentary canal caudad of the gizzard by carefully cutting the septa surrounding it and severing it in two places. Then with the aid of a hand lens study the pair of coiled tubular nephridia on the floor of each metamere. The main part of each nephridium consists of a coiled tube comprising three loops lodged in the cœlom of each segment through the ventro-lateral walls of which it opens to the exterior. The aperture is the *nephridiopore* previously noted in the study of external characters. The cœlomic opening of the nephridium is situated in the segment cephalad of the one in which the main structure is located; the tube penetrates the septum and opens into the cœlom of the preceding segment by a funnel, called the *nephrostome*. Seize a septum with the fine forceps and gently move it caudad and cephalad. It will then be possible to recognize on either side of the midventral line the two minute nephrostomes as the septum changes position.

During life the cœlomic cavity is filled with a fluid. The cilia on the nephrostome and in the beginning of the tubule create a current by means of which waste material floating in the cœlomic fluid is drawn into the nephridium and in this way eventually conducted to the exterior. The middle portion of each tubule is much thicker and has glandular walls. It functions by removing waste matter in solution from the blood which reaches the walls in a network of capillaries. The portion of each tubule near the excretory pore is dilated and probably subserves the purpose of a bladder.

On the plate above mentioned label the parts of the excretory system. Write these names on the right of the small drawing at the lower right-hand corner.

The Nervous System.—In the place where the alimentary canal has been removed identify the *ventral nerve cord* and trace it along the floor of the body wall in the midline. This cord extends throughout the length of the animal. Study its character

in the region from which the alimentary canal has been removed, noting in each metamere an enlargement, or *ganglion*, and *three pairs* of *peripheral nerves*, two pairs leaving the ganglion directly, and a third appearing to leave the cord itself cephalad of the ganglion. In the third or fourth segment note that the two halves of the cord diverge to pass around the alimentary canal as the *circumpharyngeal ring* which unites dorsally with the *cerebral ganglion*. Observe the pair of small *peripheral nerves* given off from the bilobed cerebral ganglion; these nerves divide into very fine branches as they extend forward into the highly sensitive prostomial region. Other peripheral nerves which supply the ventral side of the cephalic end of the body are given off from the circumpharyngeal ring.

Label the parts of the nervous system on both the large and small drawings of the plate.

The Reproductive System.—A complete set of both male and female genital organs occur in the same individual. That is, the earthworm is *hermaphroditic*.

By careful dissection remove the intestine from about the sixth metamere to the twentieth. Avoid cutting any organs which lie below or to the side of the alimentary canal in the region between the ninth and fifteenth metameres.

Clear away the nephridia of the thirteenth segment and look for the pair of *ovaries*, minute white bodies lying against the septum at the cephalic end, near the nerve cord. By gently waving the septum between the twelfth and thirteenth segments the ovaries which look like tiny pears fixed at their broad ends may be identified. When mature the ova drop from the ovary into the cœlom of the thirteenth segment and are passed out of the body through a pair of nephridia which have become modified into *oviducts*. The internal funnel-shaped openings of the oviducts lie in segment 13, while the ducts penetrate the septum entering the cœlom of the fourteenth segment, where they lead obliquely outward, opening to the exterior in this segment. In the wall of each oviduct is an enlargement, the *egg receptacle*, for the storage of eggs.

Associated with the female genital organs are two pairs of small white *spherical bodies*, the *seminal receptacles*, for the temporary storage of spermatazoa received from the worm with which reciprocal mating occurs. These are located in the ninth and tenth somites and open to the outside by two pairs of *spermathecal*

pores situated between segments 9 and 10 and 10 and 11, respectively.

The most conspicuous structures of the male reproductive organs are three pairs of *seminal vesicles* located in segments 9, 11, and 12, respectively, connected with two central reserviors lying in the midventral portions of somites 10 and 11. These *sperm sacs* are associated with the *testes* of the male which lie encased within the central reservoirs. By carefully peeling away the tops of the reservoirs on the right side of the animal the glove-shaped testes of that side may be exposed, one each at the anterior ends of the tenth and eleventh segments near the midventral line. Behind each testis is the enlarged *ciliated funnel* which forms the opening of the sperm duct or *vas deferens.* Trace a vas deferens back through the septum, noticing the several convolutions, and then the straight caudal extension along the floor of the body cavity to its opening, through the *spermiducal pore,* in the fifteenth segment. Note that the two vasa deferentia on each side unite in the twelfth somite.

If any difficulties were experienced in dissecting this system on the right side of the body repeat the dissection upon the left.

Label the parts of the Reproductive system on the plate of the anterior end of the earthworm.

A Cross-section of an Earthworm.—Study the cross-section first under a dissecting microscope for the plan of structure of a cœlomate animal, that is, one which possesses a cœlom. Note the relative thickness of the body wall at different levels and compare the contour of its outer and inner surfaces. Recall structures previously studied which may account for the division of the body wall into definite, well-marked areas. How many of these areas are there?

If the section studied happens to be in the proper plane the slender, curved, rodlike *setæ* already noted upon the surface may be seen. They are of the same composition as the cuticle and like it secreted by the epidermis. They may be seen projecting through the body wall. Between the body wall and the intestine is the *cœlom* within which fragmentary portions of *nephridia* may be made out. Why do the sections not show complete nephridia? Note the *intestine* with its dorsally invaginated *typhlosole.* Identify the *dorsal blood vessel* lying above the typhlosole and embedded in its large cells, the *ventral blood vessel* below the intestine, and the *ventral nerve cord* just below the ventral blood vessel.

Make a diagram, four inches in diameter, of a cross-section showing the features described above. Do not represent the cell structure.

Having obtained a general idea of the cross-section, study it with the low power or if necessary the high power for detailed structure. Note the following features of the body wall from without inward: (1) a very thin *cuticle* or non-cellular covering; (2) the *epidermis*, consisting of a single layer of cells, among which numerous *gland cells* may be distinguished by their oval shape and swollen appearance; (3) the *muscular layers*, consisting of a thinner layer of very long, slender, circular muscle cells embedded in connective tissue and attached to the septa, the *longitudinal layer* of muscle cells which when seen in cross-section appear feather-like; (4) the *peritoneum*, a thin layer of flat cells lining the body cavity and attached to the inner surface of the longitudinal muscle layer.

The intestinal wall shows the parts arranged in layers. Some of these layers, notably the longitudinal muscle and vascular layers, are inconspicuous and made out with difficulty, hence may be omitted from an elementary study. The evident layers are: (1) the *peritoneum* on the outer surface, comprising large yellow cells called the *chlorogogen* cells; (2) the *circular muscles*, a thin but continuous layer of circularly arranged muscle cells; and (3) the *lining epithelium*, or the innermost single layer of elongated ciliated cells.

In a sector of the diagram previously drawn show the details of cellular structure of the body and intestinal walls.

Nerve Cord.—The nerve cord is somewhat oval in outline and is covered externally by a sheath, consisting of peritoneum, connective tissue, muscles, and blood vessels. The *subneural* blood vessel on the midventral side and the *paired lateral neural* blood vessels, one on each side of the subneural vessel, can be distinguished under high power. In the dorsal portion of the cord are three large, clear areas, the *giant fibers*, which extend for long distances in the worm. Each is surrounded by a thick sheath. If the section happened to pass through a ganglion of the cord a number of large *nerve cells* may be present. The rest of the cord is made up of numerous small nerve fibers forming a network.

Make a drawing, one and one-fourth inches in diameter, of a cross-section of a nerve cord. Show in detail the structures described above.

PHYLUM MOLLUSCA

Many peculiarities of organization set off the animals of the phylum Mollusca from those previously studied. Molluscs possess bilateral symmetry, but a pronounced regional differentiation is lacking.

Class Pelecypoda *Unio or Anodonta* (*or Any of the Bivalves Commonly Used*).—Animals of these genera wander along the muddy bottoms of creeks, ponds, and rivers, with the anterior end buried in the mud. The food consists of minute plants and animals gathered from the respiratory current.

Exterior of Shell.—The shell comprises right and left halves (right and left valves) which retain their continuity along the dorsal side in the elastic hornlike hinge. At other points the two halves touch upon one another only. Note the *concentric lines* on the shell indicating stages in its growth. The knob on each shell near the hinge about which the growth lines are concentrically arranged is the *beak* or *umbo*. Its apex is directed toward the anterior end of the animal. Taking the position of the hinge and umbo as a basis, determine the toponomy of the clam body.

Structure of the Shell.—The shell is composed of three layers: the thin outer dark-colored layer of *conchiolin* (hornlike in appearance); the middle or *limestone layer* of perpendicularly arranged prisms; and the inner or *mother-of-pearl layer* formed of limestone arranged in sheets. Break a fragment from the edge of one valve and examine these layers.

Inner Surface of the Shell.—Lay back the left half of the shell and study the internal surface. This can be done by first opening the shell slightly. Insert a scalpel at each end of the hinge and cut the muscles which draw the halves together. The left valve must also be carefully freed from the soft parts within before the two halves are widely separated.

In Unio the valves articulate with each other by means of conical and ridged projections called *hinge teeth*. These dovetailing teeth hold the valves in their proper relations when moving upon one another. Hinge teeth are almost entirely wanting in

Anodonta. The elasticity of the hinge opens the shell. Closing the shell is effected by the action of muscles.

Notice the line on the inner surface of the shell about a half inch from the free edge of the left valve running more or less parallel with it. This is the mantle line to which the mantle was attached. The mantle is the thin sheet of tissue enveloping the body and lining the shell.

The Body (Soft Parts).—Submerge the right valve, which still contains the body of the animal, in a dissecting tray of water. Note the position of the two largest muscles, the *anterior adductor*, in the cephalic and the *posterior adductor* in the caudal end of the body. Follow these muscles to their attachments in slight depressions on the shell, termed *muscle scars*. These muscles close the shell working against the elasticity of the hinge which opens it. Manipulate the valves so as to demonstrate the elasticity of the hinge.

The body is enveloped or concealed within the *mantle* which consists of two lobes, the *right* and *left*, the posterior margins of which are thickened and somewhat modified to form the *siphons* through which water enters into and passes out of the mantle cavity (the space between the mantle folds).

To facilitate further study, remove the left mantle lobe by carefully cutting it away with scissors, leaving about an inch of the lobe in front of the siphons undisturbed, taking great care neither to remove nor to destroy the *labial palps*, which adhere closely to the mantle at its cephalic margin. This exposes the *mantle cavity* within which is suspended the muscular foot. The dorsal part of the foot and the mass toward the hinge line lodges the viscera and is termed the *visceral mass*.

On either side of the visceral mass, extending into the mantle space, is a pair of thin, striated *gills*. Raise the foot gently and observe the corresponding pair of gills on the right side.

In the posterior part of the body the thickened margins of the mantle lobes form by contact the *ventral* or *inhalant siphon*, dorsad of which is the smaller *exhalant siphon*. With these facts in mind, determine by dissection the course followed by the respiratory current.

The *mouth* is situated at the cephalic end of the body between the foot and the anterior adductor muscle. Identify the *labial palps*, which are somewhat rolled and twisted flaps associated with the mouth.

Carefully separate the mantle lobes just caudad of the posterior adductor muscle and trace the caudal course of the *intestine* which lies exposed along the caudal border of the muscle. It terminates in the *anus,* which opens into the *cloacal* or exhalant siphon at a level with the ventral margin of the posterior adductor muscle.

Make an outline drawing, each valve four and a half inches long, of the united halves of the shell with the left valve at the top of the page. In the left valve represent the inner surface of the shell and also show any external structures which may be visible from this aspect. In the right valve show as many details as possible of the soft parts discussed above. Indicate the course of the respiratory current by means of arrows.

PHYLUM ARTHROPODA

The phylun Arthropoda is the largest of all phyla, comprising about four-fifths of all the known animal species. The fundamental plan of organization has enabled these animals to compete successfully both with other animals and with the forces encountered in their environment.

Class Crustacea *Crayfish (or the Lobster May Be Used)*.—With few exceptions the animals of the class Crustacea are aquatic. Respiration is either by means of gills or directly through the surface of the body.

The common crayfish is an inhabitant of fresh-water streams and lakes.

External Structure.—Study a specimen in a dissecting pan. The hard external covering, the exoskeleton, is secreted by the ectoderm and corresponds to the cuticle of the earthworm. It is composed of *chitin* and serves for *protection*, *support*, and the *attachment* of muscles. As this covering cannot expand with the growth of the animal, it must be molted (shed) periodically.

Regional differentiation is pronounced as attested by the presence of head, thorax, and abdomen in each of which the component segments resemble each other more than they do those of other regions. In the crayfish and others of its kind the head and thorax are outwardly united. The line of demarcation is a groove over the sides and dorsum of the shell and known as the *cervical* or *neck groove*. When head and thorax are thus united it constitutes a *cephalothorax*. The single piece of exoskeleton covering this portion of the body dorsally and laterally is known as the *carapace*.

The head terminates in the anterior pointed extension of the carapace called the *rostrum*. This division of the body is provided with a number of sense organs more complex than those found in forms previously considered. Notice the pair of large *compound eyes*. These are stalked and movable and are considered by some to be appendages. In front of the eyes are the smaller first sensory appendages or *antennules*. Just below are the long, flexible, many-jointed pair, the *antennæ*. Both pairs

of sensory appendages render services which are tactile and chemical in nature. Note the openings of the *nephridia* (green glands) on the middle of the ventral surface of the basal segment of the antennæ.

The cervical groove, already referred to, crosses the dorsum of the carapace at about the middle of its extent and is thence continued forward on either side as a groove, which ends just laterad of the antennæ. The cervical groove marks the boundary between head and thorax. The ventro-lateral edge of the carapace is not attached, the free plate on each side being known as the *gill cover*, since underneath are found the gills by means of which these animals obtain oxygen.

When the animal is viewed from the dorsum, those appendages constituting the mouth parts (serving as jaws and conveyors of food to the mouth) may be seen projecting slightly beyond the cephalic margin of the carapace. These appendages named in order from the first or cephalic pairs are: mandibles, one pair; maxillæ, two pairs; maxillipeds, three pairs. Caudad of these and borne by the thorax are five pairs of walking legs the anterior pair of which is modified into large pincers termed *chelæ*. The basal piece of the third pair of walking legs bears the genital opening in the female. The fifth pair of walking legs in a corresponding location bears the opening of the male genital organs. The appendages will be studied in more detail later.

The abdomen comprises seven segments six of which bear appendages. These appendages are called *swimmerets*. In the female they serve as attachments for the eggs which are thus carried during the developmental period. The first pair of abdominal appendages in the female are reduced. In the male they are fused to form an organ for the transfer of sperm to the female. In the sixth pair of swimmerets, called the *uropods* (meaning "tail-foot"), the parts are greatly enlarged and form with the *telson* (last segment bearing the anus) an efficient swimming organ, termed the *caudal fin*.

On the appropriate plate label all the above-described structures, which are visible from the dorsal side.

The Gills.—Remove the carapace of the left side and note the pinnate (feather-like) gills underneath. Study those above the second or third walking leg. The gill exposed at the base of the appendage is the *podobranch* (foot gill). In the lobster the delicate lamina uniting with its base and forming the division

line between the gills of successive segments is known as the *epipod*. This is very much reduced in the crayfish. Turn the epipods so as to expose the two *arthrobranchiæ* (joint gill) which are fixed to the flexible interarticular membrane connecting the basal joint of the leg to the thorax. In the lobster slightly dorsad of the arthrobranchiæ may be found the *pleurobranchiæ* (thoracic wall gills) which grow out from the sides of the thoracic walls.

Examine the region where the gill cover was cut away and note that this extension of the carapace over the branchial chamber is not the true sides of the thorax but rather an outward and downward growth from the carapace. The gills conceal the true body wall.

Slice off a portion from the tip end of the eye stalk, including only the outer pigmented covering. Remove the pigment from the under surface and mount the transparent piece (cornea) in water on a slide.

Make a sketch of several corneal facets on the plate of the dorsal aspect of the animal.

The balancing organs or *statocysts* are located in the dorsal portion of the basal segment of the antennules. They consist of a pocket or invagination of the surface, which is lined with sense hairs, among which solid particles are lodged.

Remove the right antennule and cut off the ventral wall of the basal segment. Investigate the saclike statocyst, determining its relations, contents, and the nature of its lining.

Many fine hairlike processes of chitin on the body and appendages are of a tactile nature, while others on the antennules, antennæ, and mouth appendages provide a gustatory service.

The Appendages.—The 19 pairs of appendages exhibit considerable diversity of structure in different segments of the body; yet all possess the same structural plan and in early stages of development are quite similar. Among these appendages there is a close correlation between structure and function, a fact which bears witness to their value as good material for the study of serial homology.

Typical Segment and Pair of Appendages.—As the abdominal segments are most distinct and less modified than those of other regions, the *third* or *fourth*, with its appendages, may be studied as a typical somite. Although the regions of the exoskeleton constitute a continuous whole, it is convenient to speak of the

convex dorsal portion as the *tergum* (meaning the "back"), the lateral lobes as the *pleura* (singular, *pleuron*, meaning the "side"), and the slender ventral bar as a *sternum* ("breastbone").

By removing the right appendage the following parts may be made out: Its union with the segment is through a division called the *protopod.* This consists of two portions, a very small basal part, the *coxopod*, and a long distal part, the *basipod.* From the basipod arise two flattened, many-jointed plates. The outer of these is the *exopod;* the inner one near the median line, the *endopod.*

This type of appendage found on the abdominal segments is called the forked or *biramous* type of appendage. It is regarded as the most primitive arthropod appendage, all other kinds having been derived from such an appendage through modifications.

The Remaining Abdominal Appendages.—Remove the remaining appendages from the left side of the abdomen and arrange them in the order of the segments to which they belong, beginning at the cephalic end. The appendages of the second to fifth somites, inclusive, in both sexes are quite similar to those of the typical segment described above. There are the usual two joints of the *protopod* comprising a short *coxopod* and a long cylindrical *basipod.* Both the *endopod* and *exopod* are present as many jointed filaments, the former slightly longer than the latter. The *uropod* comprises a very broad and thick single-jointed *protopod* and two terminal oval plates which represent the endopod and exopod. The latter is divided by a transverse suture into two pieces. Noticeable differences exist between the appendages of the first abdominal segment of the male and female. In the male these appendages are stiffened and otherwise modified so as to form an organ for transferring sperm to the proper receptacle in the female. The first abdominal appendage of the female is flexible and much reduced.

Although wide variations in the appendages of the thorax and head exist, they are nevertheless reducible to the same fundamental plan of structure as the abdominal appendages.

The Appendages of the Thorax.—Beginning with the last pair of walking legs, remove eight appendages from the left side. Be sure to cut these close to the body wall so as not to destroy their *podobranchiæ.* The thoracic appendages include five pairs of walking legs and three pairs of smaller appendages, the *maxillipeds.* Arrange these appendages in serial order for study.

The *third maxilliped* is one of the most complete of these appendages and will be used as an approach to the study of the whole series. Compare with a swimmeret. Identify the parts, note differences, and determine the type. Express the results in writing and submit as a part of the laboratory report. These comparisons may be expressed in the form of a table.

The *second maxilliped* possesses essentially the same components as the third, but the *exopod* is relatively larger, the *endopod* smaller.

In the *first maxilliped* the coxopod and the basipod are broad, thin plates, while the *endopod* is short and only two-jointed. The undivided portion of the exopod is very long. In place of the podobranchia is a broad membranous plate.

Examine the left *third walking* leg and compare with the third maxilliped. This walking leg has a *coxopod* with an *epipod* and a *gill*, a *basipod* and an *endopod* of five joints. The *exopod* is lacking, and therefore this more specialized unforked type is known as a *uniramous appendage*. Note the pincer formed by the prolongation of one angle of the distal end of the penultimate joint, thus forming a fixed jaw for the last joint to work against.

Examine the other walking legs. Do all have pincers, epipods, and gills? The first pair is greatly enlarged, with a powerful pincer for offensive and defensive use.

Draw the left third maxilliped and left third walking leg in the same relative positions.

The Appendages of the Head.—Remove the remaining appendages of the left side and arrange in a series for study and comparison.

The *coxopod* and *basipod* of the second maxilla are still thinner and more lamellate than is the case of these parts in the first maxilliped and are subdivided by deep fissures which extend from their inner edges. The *endopod* is very small and undivided, while the so-called *bailer* is probably formed by a fusion of the *exopod* with the *epipod*. The bailer moves in such a manner as to draw water through the branchial chamber over the gills from the caudal end.

In the first maxilla the *exopod* and *epipod* have disappeared, and the *endopod* is insignificant and unjointed. Its two inner thin plates are the *protopod*. The small process in front of this appendage in the head is not considered as an appendage but as a part of the lower lip.

The *coxopod* of the *mandible* is greatly elongated transversely to form a strong functional jaw. The *basipod* possesses a much reduced two-jointed *endopod* which forms the palp. The *exopod* is lost. In front of the mouth opening is the upper lip or *labrum*.

The *protopod* of the antenna is two-jointed, the *coxopod* being small and bearing the aperture of the duct of the renal gland, while the basipod is larger. The long many-jointed filament connected with the basipod by two stout basal segments is the *endopod*. The thin, sharp projection near the base of the filament is the *exopod*.

Draw the left antenna enlarged about three times.

The *antennule* possesses a three-jointed protopod, from which arise two, short, many-jointed filaments. These may or may not represent the *endopod* and *exopod*. It is perhaps doubtful whether this appendage is strictly comparable with the others.

This study shows that there are at least 5 pairs of appendages borne upon the head, 8 upon the thorax, and 6 upon the abdomen, making a total of 19. If the eye stalks are construed as belonging to the same category of appendages as the others and, therefore, representing a segment, and the telson accorded the rank of a segment, then there are represented in the body of the crayfish 21 segments.

Class Insecta: *The Lubber Grasshopper, Schistocerca americanum* (*or an Allied Species*).—One of the Southern species of grasshopper is chosen for study because of its large size. Most of our grasshoppers, so common in the summer and autumn, show the same fundamental plan of structure but are difficult of study because of their small size.

External Structure.—Study a specimen in a dissecting pan. A chitinous exoskeleton, lacking the lime deposits of the crayfish, is present and is secreted by the ectoderm lying beneath. The hard surfaces of the exoskeleton are known as *sclerites*, which are joined by *sutures* less densely chitinized.

Regional Differentiation.—Note that the bilaterally symmetrical body is divided into three distinct regions; the movable *head*, the *thorax* bearing legs and wings, and the conspicuously segmented *abdomen*.

The Head and Its Appendages.—The head exhibits no clear evidence of segmentation. The six segments (at least) which it comprises are suggested by comparative structural studies. The larger part of the head is enclosed in a single sclerite, the

epicranium, in which the *dorsum* is spoken of as the *vertex*, the sides as the *genæ*, and the front as the *frons*. The frons extends ventrad to a distinct *transverse suture*, below which is a very broad sclerite known as the *clypeus*. Attached to the ventral border of the clypeus is a broad, freely moving flap, the upper lip, or *labrum*. This is not a true appendage but a part of one of the segments which enters into the composition of the head skeleton.

The head bears eyes, antennæ, and three pairs of mouth parts. Note the two large, brown, oval areas on the dorso-lateral portions of the head. These are the *compound eyes*. Remove a portion of the cornea, clean out the pigment, and mount in water on a slide. Are the facets of this cornea similar in shape to those of the cornea taken from the eye of the crayfish? The grasshopper also has three simple eyes, or *ocelli*, one in the depression of the middle of the frons, the other two cephalad of the dorsal portion of the two compound eyes. The *antennæ* are many-jointed appendages springing from the space between the compound eyes. Raise the upper lip and note a pair of heavy, black *mandibles*. Determine the direction of their movements. Just caudad of the mandibles is the first pair of *maxillæ* with feelers or palps at the sides, and lying back under the head, bearing palps on either side, is the second pair of *maxillæ* which have fused with one another to form the *labium* or lower lip. The antennæ and mouth parts of the grasshopper represent modifications of typical arthropod appendages.

The Thorax and Its Appendages.—The thorax comprises three segments, called the *prothorax*, *mesothorax*, and *metathorax*, when named in order from the cephalic end. These divisions are easily recognized by the appendages which they bear. One pair of legs is attached to the ventro-lateral margin of each thoracic segment. In addition, the mesothorax and metathorax each bears a pair of wings articulated with the dorso-lateral margin.

Remove the wings from the left side and compare them. What are your deductions? The wings arise as saclike evaginations of the body wall. During development the two walls of the sac become pressed together, forming a thin membrane. The *veins* of the wings are respiratory tubes or *tracheæ*, filled with air, each surrounded by a tubular blood sinus. After the insect attains its full size, blood ceases to flow in the wings and they become the dry, hard, and lifeless structures of the adult.

All three pairs of legs of the grasshopper are used in walking, but the third pair are primarily jumping legs and show structural modifications for such a use. Remove a walking leg from the right side of the body and study. It has five parts. The short joint next to the body is the *coxa*. The second segment or *trochanter* is smaller than the coxa, its ventral aspect being longer than the dorsal. This segment is succeeded by the long *femur*. Distad of this is placed the slender, spiny *tibia*, while the *tarsus* includes all that part of the leg distad of the tibia. It consists of three segments. The last segment of the tarsus bears a pair of claws and a single cushion or *pulvillus*. In addition there are four pairs of pulvilli under the other segments of the tarsus. Compare the jumping leg with the other walking legs as to relative proportions of parts and direction of the convexities of the articulations.

In the membrane along the sides of the thorax, just cephalad of the mesothorax, is a small opening. This is the *mesothoracic spiracle*, one of the openings of the respiratory system. Notice also on the pleural suture which separates the mesothorax from the metathorax a similar opening, the *metathoracic spiracle*. Both openings are guarded by lips.

The Abdomen and Its Appendages.—Each complete segment of the abdomen consists of an inverted U-shaped *tergum* bearing on its ventral border a slight indentation, the *abdominal spiracle*. This arched plate is probably a fused tergum and pleuron. There is a convex ventral plate, the *sternum*. The rest of the *pleuron* is represented by the membranous fold where tergum and sternum articulate.

The sternum and tergum of the first abdominal segment are separated from each other by the depression for the insertion of the third pair of legs. The tergum of this segment bears upon either side a kidney-shaped opening, covered by a thin, semi-transparent membrane. This is the *chordotonal organ*, erroneously called an ear. On the anterior border of the chordotonal organ is the first abdominal spiracle. How many spiracles are present on the body of the grasshopper?

The next seven segments are alike in both sexes but the remainder of the abdomen is different in form and structure in the male and female. In both sexes the ninth and tenth terga are narrow and partially fused on their lateral margins. The eleventh tergum or *suranal plate* extends caudad forming the roof

of the anal opening. On each side, projecting from beneath the caudal border of the tenth tergum, is a pointed appendage termed the *cercus* (plural, *cerci*). These are generally regarded as the modified appendages of the tenth segment. Each cercus partially covers a much larger, triangular sclerite which extends from the lateral border of the tenth tergum caudad. These are the *podical plates.*

In the male the broad ninth sternum is followed by a hood-shaped tenth which forms the caudal end of the body and terminates dorsad in two points. This is commonly called the *subgenital plate* as it forms a hood about the genital apparatus.

The most prominent portion of the caudal part of the abdomen of the female is the *ovipositor,* composed of two pairs of rigid and pointed pieces and employed in forming a hole in the ground within which the eggs are deposited. Between the ventral pair is the opening of the oviduct. From the median caudal margin of the eighth sternum a pointed projection, *the egg guide,* extends caudally between the ventral pieces of the ovipositor and aids in placing the eggs.

On the plate of the left lateral aspect of the female indicate by means of brackets and labels the three divisions of the body. These should be placed above the drawing. Label the various external structures described for each region. The abdominal segments may be numbered serially. The abdominal terga and sterna may be designated by symbols, as, for example, t_2 referring to the tergum of abdominal segment 2, and s_2 referring to the sternum of abdominal segment 2.

Secure an animal of the other sex, study and label the structural modifications of the caudal end.

It will be profitable to devote some time to a comparison of the figures of the crayfish and grasshopper with regard to body regions and appendages including types, distribution, modifications, and functions. Such a study might be summarized in the form of a table.

PHYLUM CHORDATA

The phylum Chordata, which is the last in the series, comprises animals which are diverse in form and structure. All are alike, however, in the possession of certain fundamental characteristics of organization which are not found combined in any other animals. The phylum is divisible into four subphyla, a representative of one of which will be studies by way of gathering first-hand information regarding the general plan of organization which prevails throughout the phylum.

Subphylum Cephalochorda *Branchiostoma* (*Amphioxus*).—This subphylum comprises about a dozen species of small marine animals called "lancelets." They possess in a typical state the fundamentals of organization common to all chordates. These animals are found near the shore, where they burrow in the sand which may be entered either head or tail foremost. The body, with the exception of the anterior end, is usually concealed by day, but the animals may leave their burrows at night and dart through the water. While rapid and powerful swimmers, they topple over upon their sides when forward progression ceases.

External Structure.—Study a stained and mounted specimen of a young animal under the dissecting microscope or low power of the compound microscope and note the spindle-shaped body and the lack of a well-defined head. Along the middorsal line note the low, nearly transparent fold extending the entire length of the body. This fold is the *dorsal fin* which becomes broader in the tail region and is continued around the end of the tail to the ventral side where it narrows in its cephalic extent. This broader portion of the fin fold about the end of the tail is known as the *caudal fin*, while the narrower ventral part extending cephalad from the caudal is the *anal fin*. Two ventro-lateral folds, the *metapleural folds*, border the flattened ventral surface of the anterior two-thirds of the body and give this portion a triangular shape when viewed in cross-section. These may be seen in preserved specimens.

The sides of the body are marked by a series of V-shaped lines, formed by *myosepta* of connective tissue which divide the great

longitudinal muscle mass into segments called *myotomes* or *myomeres*.

Locate the opening of the oral hood, which is a large oval aperture on the ventral surface of the cephalic end of the body. It is fringed on each side with about 12 ciliated tentacles or cirri, known as the *oral cirri*. The *anus* is on the left side of the anal fin just at the base of the caudal fin. The *atriopore* is a median aperture on the ventral surface between the cephalic end of the anal fin and the convergence of the metapleural folds. The respiratory current entering the mouth leaves the body through this pore.

Internal Structure. *The Digestive and Respiratory Organs.*—The alimentary canal is essentially a straight tube. The oral hood and cirri have been mentioned above. The walls of the oral hood delimit a space called the *oral cavity*. The anterior part of the enteron is the *pharynx*, the word here being used to mean not merely a throat but a division of the enteron which serves the double purpose of conveying both the respiratory medium and the food. It is a compressed tube, occupying about one-half the total length of the alimentary canal. It is surrounded by a space, the *atrial cavity*, which will be seen in its proper relations in the study of a transection of this region. The pharyngeal walls are perforated by numerous slitlike apertures, the *gill slits*, running obliquely ventrad and caudad. Water taken in at the mouth passes through the gill slits into the atrial cavity from which it escapes through the atriopore. The epithelium lining the gill slits constitutes the respiratory tissue.

Between the cavity of the oral hood and the beginning of the pharynx there is a circular sheet of tissue which appears as a straight line when viewed from the side of the animal. This membrane is called the *velum*. It is perforated by the mouth opening.

Caudad of the last gill slit the alimentary canal narrows and continues as the intestine which, a short distance caudad of the pharynx, gives off from the ventral side a blind pouch. This evagination is the *liver* the blind end of which extends cephalad on the right side of the pharynx.

The Skeleton.—The skeleton is of the endoskeletal type and extreme in its simplicity. It consists of a flexible, rodlike structure above the alimentary canal and extending throughout the entire length of the body. This is the *notochord* which serves

as an axial skeleton. Other skeletal parts are found supporting the oral cirri, gill slits, and mesal fin fold.

* *The Central Nervous System.*—The brain and spinal cord lie immediately above the notochord. Determine the extent and relations of the central nervous organs.

Upon the plate of the left lateral aspect of Branchiostoma add features of external and internal structure which have been observed in the above study.

Study of Transverse Sections.—The relations of internal organs are more readily understood through a study of transverse sections. For this purpose prepared slides through the region of the pharynx and liver are most instructive.

Use a low-power microscope to determine the shape and general location and relations of internal structures. A higher magnification may be used for the study of details. An explanation of the stains used in preparing the slides will be given by the instructors.

The section chosen for description here is one passing through the body at the level of the liver. In section the body is triangular in shape. In the midvertical portion may be seen the *compressed pharynx* on the right of which is the *liver*. The *gonads* on either side appear as elevations from the walls of the body encroaching upon the *atrial cavity*.

The *epidermis* consists of a single layer of cells supported by an underlying thin layer of connective tissue, the *dermis*.

Identify and locate *pharynx*, *notochord*, and *spinal cord*. Determine the following: (1) the nature of the notochordal cells, notochordal sheath, and the gill slits; (2) the nature of the spinal cord; (3) the nature and relations of muscle segments (myomeres); (4) the nature and relations of muscle partitions (myotomes); (5) the dorsal groove of the pharynx (*epipharyngeal groove*) and the ventral groove (*endostyle*). These grooves serve to entangle the minute particles of food and transport them toward the intestine.

The cœlom is much reduced, having been encroached upon by the extensive atrial cavity. The remnants of the cœlom consist of paired cavities at the sides of the epipharyngeal groove, a single space ventrad of the endostyle, a space between the walls of the liver and the atrial epithelium, and the space surrounding the gonads.

The larger blood vessels can often be recognized by the coagulated blood which they contain. The *dorsal aortæ* comprise a pair of vessels lying one on each side of the epibranchial groove,

while the *ventral aorta* (or heart) is a median vessel lying in the endostylar cœlomic canal. Along the dorsal surface of the liver in the cœlomic space between the liver and the atrial membrane may be seen three or four *hepatic veins* lying side by side, and in the cœlomic cavity surrounding each gonad a more or less prominent blood vessel is located.

The *reproductive organs* occupy similar positions in the two sexes. The sections show them extending toward the atrial cavity from the region of the atrial folds. If available, examine demonstration section through ovaries containing ripe eggs.

The *liver* is located at the right of the pharynx and appears to lie free within the atrial cavity between the pharynx and the gonad. Its walls are thick.

Upon the outline drawing of the transection represent the structures which are missing. Certain structures should be represented in colors as follows:

Nervous organs, green.
Notochord, brown.
Celom, yellow.
Connective tissue, blue.
Blood vessels, red.
Muscle, stippled with black.
Gonads, purple.

SUBPHYLUM VERTEBRATA

This subphylum includes all chordates which possess: (1) a segmented vertebral column, (2) a heart with at least two compartments, and (3) a cranium.

Since an extended study of a vertebrate, the frog, has already been made, the object of the present study is that of affording some direct contact with the lower or fish-like vertebrates.

Class Elasmobranchii. *The Spiny Dogfish, Squalus acanthias (or Any Shark).*—Sharks and rays constitute this class of fishlike vertebrates. In a general way they resemble the true fishes but differ from them so widely in structure that they are to be regarded as constituting a separate class.

This species is common along the Atlantic Coast of New England. Preserved specimens retain their natural coloration and body form to a remarkable degree. The adults are about three feet long. Study a preserved specimen on a dissecting tray. It should be thoroughly rinsed and kept moist during the progress of study.

Exterior.—The body form is typical of aquatic vertebrates, being spindle shaped, with the deepest part between the first and second thirds of the total length. This so-called "stream-line" form is the most advantageous for progress in a liquid medium.

The body is divided into *head*, *trunk*, and a portion caudad of the cloacal aperture termed the *tail*. Such a morphological tail is not found outside the chordate phylum. The skin is beset with minute *placoid scales* each of which bears a tiny *spine*. The resistance encountered in passing the finger from the tail toward the head is due to the caudally projecting spines of these scales. Embedded in the dermis is a bony plate from which the pointed spine of *dentine* projects through the skin. The spine is covered with *enamel*. View the surface of the skin with a lens.

A light-colored line, the *lateral line*, extends along the sides of the body from the head to the tail. Its location along the side of the body suggested its name. In structure the lateral line is a canal embedded in the epidermis. It communicates with the exterior through pores. Within the lining of the canal there are

sense organs whose function is the detection of disturbances in the water. The lateral-line canals are continuous with similar canals upon the head. Together these constitute the lateral-line system of sense organs.

Study the surface of the animal with special reference to the following features: (1) shape of head; (2) mouth, jaws, and teeth; (3) nostrils; (4) eyes and the state of lids; (5) external evidence of ears; (6) pores of *endolymphatic ducts*, one on either side of the middle line of the head just caudad of the eyes, communicating with the inner ears; (7) gill slits, their numbers, relations, and the location of the functional respiratory tissue; (8) spiracles, caudad of the eyes, and their relations; (9) the mesal or unpaired fins comprising a *first* and *second* dorsal and a *caudal* or *tail fin* the relation of which to the end of the body should be determined, this relation being known as that constituting the *heterocercal tail;* (10) the *paired fins*, those of the shoulder region called the *pectoral fins*, those of the hip region, the *pelvic fins*. In male dogfishes, the mesal side of each pelvic fin is modified into a stout process called the *clasper* which is used by the males as an intromittent organ while transferring the seminal fluid to the females. All the fins possess flexible supports called the *dermal fin rays*, embedded in the skin of the fins.

Draw the left lateral aspect, labeling all the parts mentioned above as far as they may be seen from the exterior.

If time permits, a further study of the shark may be made independently.

Class Pisces.—The class Pisces includes aquatic vertebrates which in numerous ways are readily distinguished from elasmobranchs (sharks and rays). In this connection reference might be made to the presence in fishes of an operculum or gill cover concealing the gills and gill slits. Within the class one may recognize four subdivisions. The most numerous and widely spread of the piscine types constitute the subdivision called the *Teleostei* or bony fishes, since the skeleton is formed almost entirely of bone.

Although the directions which follow refer specifically to the yellow perch (*Perca flavescens*), they may be used in the study of almost any bony fish. Most of the observations can be made on preserved material, but it is desirable that observations be made also upon living specimens during the progress of the laboratory study.

External Features.—Observe carefully the form of the body with a view to expressing verbally not only its nature but also its adaptation to a darting mode of locomotion through a liquid medium. Hold the fish on a level with the eyes, the tail pointing *straight away* from the observer's face. What is the nature of the visible surface? The body may be recognized as possessing head, trunk, and tail regions. The *scales* are flat and imbricated and are arranged in diagonal rows.

The *mouth* is terminal in location. On the dorsal surface of the head immediately in front of the eyes is a pair of small openings on each side. These are the *nostrils*. The two openings of the same side lead into a common *nasal cavity*. Water enters by one aperture and leaves by the other. Look for the *eyelids*. Only that part of the ear mechanism corresponding to the frog's inner ear is present in fishes. Accordingly, there is no external indication of the presence of this organ, which is located just caudad of the dorsal portion of the eyes.

On the sides of the head note the gill cover or *operculum* composed of flat bones overlaid by a thin layer of soft tissues. By elevating the operculum the gills and gill slits may be seen. Attached to the ventral margin of each operculum is a membrane, the *branchiostegal membrane*, supported by skeletal elements called *branchiostegal rays*. To see these the membrane must be spread.

In order to study the respiratory structures to a better advantage remove the operculum of the left side. Expose the mouth cavity and pharynx by cutting through the angle of the jaws and extending the cut across each of the supporting arches of the gills. Divaricate the parts thus severed.

The supports of the gills are known as *gill arches*. They, together with the jaws and the tongue support (hyoid arch), constitute the *visceral skeleton*. Between the gill arches are the *gill slits*. The pharynx or posterior portion of the exposed cavity can be distinguished from the *oral* or mouth cavity by the presence of the gill arches and gill slits in its walls.

Of the five gill arches only the first four actually bear gills. The dorsal ends of the arches of each side are attached to the undersurface of the brain case, while the ventral ends articulate with a series of bones in the midventral line. This connecting series of bones constitutes what are known as the *basibranchials*. The inner surface of each of the first four gill arches bears spine-

like processes known as *gill rakers*. These appear in two rows and serve to prevent food from passing through the gill slits. On the outer surface of each arch are numerous *gill filaments* supported by *gill rays*. The double row of filaments form the respiratory part of the gill. Remove a portion of the first gill-bearing arch and examine it in water with a microscope.

If live fishes are available, study the successive respiratory movements.

There are many areas in the mouth and pharyngeal cavities bearing teeth. On the upper jaw teeth are borne by the *premaxillary bones*. In the center of the anterior portion of the roof of the mouth are the *vomerine* teeth. The palatine teeth extend obliquely caudad from the vomerine group. Further caudad in the roof of the pharynx are two areas, the upper pharyngeal teeth, supported by the dorsal portion of the gill arches. In the lower jaw there are teeth on the *dentary bones* which form the greater portion of the margin of this jaw.

Three structural features of the oral and pharyngeal cavities of land vertebrates are lacking in fishes, namely, the internal nares, the eustachian tube, and the glottis. Account for their absence.

Extending along each side of the body and parallel with the contour of the back is a line nearer the dorsal than the ventral side. This is the lateral line. It consists of a row of sense organs which serve to detect disturbances in the water. These sense organs lie embedded in the lining of a canal found underneath the line which shows at the surface and is connected with the exterior by minute pores which penetrate the scales covering the lateral-line canal. Remove a scale from the lateral line and examine it with the microscope for a view of such a pore.

The fins are of two types, *median* and *paired*. Of the former there are two dorsals along the midline of the back, one *caudal* (tail fin) at the caudal end of the body, and an *anal fin* at the base of the tail on the ventral side. The paired fins correspond with the fore and hind limbs of terrestrial vertebrates. The more cephalic pair is known as the *pectoral fins*, located one on each side immediately behind the operculum. The *pelvic fins* appear on the ventral surface at a level just caudad of the pectoral fins. All the fins are supported by bony structures termed *fin rays*. These are usually soft and flexible but may be spinelike as in the first dorsal fin.

Hold the fish again on a level with the eyes as before mentioned. What is the relation of dorsal and pectoral fins with regard to their surfaces which are presented to the water? With a similar object in view, observe the eyes, mouth, and other parts.

If time permits and living specimens of any species are available, observe the movements of the fish in order to determine the important organ of locomotion. Describe the method. By means of rubber bands slipped over the body, bind the pectoral fins close to the sides. Describe results. Perform the same experiment with the pelvic fins. Bind both pairs close to the body at the same time. Results?

Look on the midventral line just cephalad of the anal fin for the *anus* or opening of the intestine to the exterior. Behind this is a depression in which the *urogenital papilla* is located. This papilla bears the small openings of the urinary and genital organs.

Study more carefully the scaly armor of the fish. Note the relation of one scale to another and the difference in their size in different parts of the body. Scales are formed by and set into pockets of the dermis of the skin, the epidermis being reduced to a thin film covering the free part of each scale. With forceps remove a scale and note the scalloped margin which was set in the dermal pocket. Examine a scale mounted in water on a slide, using the low power of the compound microscope. Observe the concentric markings or lines of growth. Knowing that those lines formed during the winter season are closer together than those formed during the summer period, determine the approximate age of the specimen. Note the rows of minute denticles and the film of epidermis upon the exposed portion of the scale. Owing to the toothed border this type is known as a *ctenoid* scale.

The external fin rays, also called *dermal rays*, are formed by the dermis of the skin. Mount one of these in water on a slide and examine under the microscope. Note that the rays are composed of numerous small joints and that they branch toward the free end. Are the spiny fin rays jointed or branched?

On the plate illustrating the structural features of the perch label the nostrils, the branchiostegal membrane, a gill arch, indicating its numerical position, a gill slit, raker, and gill. Also the lateral line, fins, the dermal fin rays, the anus, and the urogenital depression.

On the same plate draw and label a scale showing lines of growth. Also a scale removed from the lateral line showing the pore to the lateral-line canal.

Internal Anatomy. Musculature.—Remove enough of the skin at the base of the tail to expose the thick layer of muscles within. This layer is divided into zigzag bands of muscle tissue. Each band is called a *muscle segment* or *myotome*. Each myotome extends from middorsal to midventral line and is separated from the adjacent myotomes by connective tissue partitions, called *myosepta*. Immediately below the lateral line is a longitudinal partition separating the myotomes into dorsal and ventral portions.

This arrangement of muscles reminds one of the state in Amphioxus and is the primitive vertebrate plan from which the muscular system of all land vertebrates is derived. It consists essentially of a longitudinal series of muscle segments. It is evidence of the segmental plan of structure of the vertebrate body.

Skeleton.—It will be recalled that in Amphioxus the skeleton consists chiefly of an elastic rod, the notochord; in the shark a more extensive endoskeleton is present and formed entirely of cartilage. In the teleostean fishes cartilage has, for the most part, been replaced by bone. This is the reason for referring to them as the "bony fishes." During development the vertebral column replaces the notochord, although vestiges of the latter persist.

Reference to a mounted skeleton reveals the same general divisions that occur in the skeleton of a land vertebrate. These are an *axial skeleton* comprising the *skull*, *vertebral column*, and the supports of the *unpaired fins* and an *appendicular* skeleton comprising the supports of the *paired fins*.

Looking further into this internal framework we find the skull divided into three main divisions, the *cranium*, the *sense capsules*, and the *visceral skeleton*. These parts which are separate during development become united in a rather compact whole in the adult.

The cranium is that portion of the skull which houses the brain. The sensory capsules are skeletal parts which enclose or support the three pairs of sense organs of the head, the nasal sacs, the eyes, and the internal ears. The visceral skeleton has already been described and studied.

Note how the bones which support the dorsal and anal fins alternate with the neural and hæmal spines. Note especially the

vertebral relations of the supporting structures of the caudal fin.

The dorsal ends of the *pectoral girdle* are attached to the caudal end of the cranium. The arch is suspended ventrally to support the pectoral fins. The *pelvic girdle* of the perch is composed of two flat plates of bone on the midventral line free from other skeletal parts.

Abdominal Viscera.—Make an incision through the body wall along the midventral line from a point immediately in front of the anus to the region of the lower jaw. Care should be used that the internal organs be not injured by too deep a cut. Make a dorsal incision at the point of origin of the midventral slit. Remove a portion of the body wall of the left side so as to expose the *abdominal cavity.*

Note the glistening membrane, the *peritoneum*, which lines the cavity and suspends the various visceral organs from the dorsal body walls. A *transverse septum* forms the anterior wall of the abdominal cavity. In front of this septum is the *pericardial cavity*, lined with a thin membrane, the *pericardium.* It contains the heart.

From the pharynx the alimentary canal continues caudad into the *œsophagus.* The œsophagus merges into *stomach* the constricted caudal limit of which is the *pylorus.* Beyond this point the canal is continued as the *intestine.* In the region of the pylorus there are three finger-like blind pouches arising from the intestine and called *pyloric cæca.* Note the *liver* attached by the *coronary ligament* to the transverse septum. The *gall bladder* lies on the posterior surface of the liver. The *spleen* appears as a small dark body between the coils of the intestine. Because of its diffuse nature the pancreas is difficult to find. A description of this organ is therefore omitted. In order to view the other viscera to the best advantage it will be necessary to strip off the layers of fat in which they are embedded.

The *reproductive system* consists of a pair of *gonads.* There is no connection between the *testes* and the kidneys as is the case in the males of most vertebrates, but the testes as well as the *ovaries* merely taper down to a tube leading to the *urogenital opening.*

Along the dorsal wall of the abdominal cavity extends a large gas-filled sac, the *swim bladder* or *air bladder.* By slitting the wall of the bladder the extent of this sac can be determined.

In some fishes the swim bladder still retains connection with the pharynx, from which it arises, but this connection is entirely lost in the perch. It is a hydrostatic organ equalizing the weight of the animal and the water it displaces. In life the swim bladder is filled with gases. By reducing the volume of gas the fish is able to descend to greater depths; by increasing the volume it is able to rise easily in the water.

Immediately dorsad of the swim bladder and just below the vertebral column are two dark-colored, irregular masses extending cephalad as far as the œsophagus. These are the *kidneys*. The *ureters* extend the whole length of the kidneys and pass along the posterior wall of the abdominal cavity to the *urogenital papilla*. Before reaching the papilla, the two ducts unite and then enlarge to form a *urinary bladder* located immediately above the caudal end of the gonads. Extend the midventral slit caudad on one side of the anus. This will facilitate the study of the caudal portions of the urogenital organs.

The Circulatory System.—The *heart* of bony fishes consists of but a single *auricle* and *ventricle*. The ventricle is readily identified as the pale, thick-walled ventral chamber, with the thin-walled, dark-colored auricle overlying its anterior portion. Posterior to the auricle is the *sinus venosus*. From the ventricle the blood passes cephalad into an enlarged vessel, the *bulbus aortæ*, and thence into the *ventral aorta* which divides to form four pairs of *afferent branchial arteries*. Each of these follows the course along a gill arch to break up into the capillaries of the gill filaments where an exchange of gases takes place. These capillaries unite into *efferent branhcial arteries* which come together on the middorsal line to form the principal artery of the body, the *dorsal aorta*. This is the single type of circulation.

A REVIEW OF THE PHYLA, SUBPHYLA, AND CLASSES OF ANIMALS

The following study is outlined as a review of the animal groups through the use of a key for their identification. The key is designed to include the forms which are ordinarily used in laboratory studies or which one might ordinarily encounter in general studies.

Unlabeled representatives of the major animal groups should be provided in numbered jars. Specimens preserved in alcohol should be dipped in water and kept moist while used for study. After each specimen is identified, write upon a record sheet the name of the group or groups to which the animal is judged to belong. Make these records according to the number borne by the specimen.

Key for the Identification of the Larger Groups of Animals

A. The body of the organism comprising a single independent cell or many similar and independently functioning cells associated in a colony with little or no differentiation (*i.e.*, without forming tissues); or comprising masses of multinucleate protoplasm; mostly microscopic
Phylum *Protozoa*

AA. The body of the organism comprising many cells of different kinds (*i.e.*, forming tissues).

B. Body usually forming irregular masses without apparent symmetry but the masses sometimes cylindrical, goblet shaped, or vase shaped, or definitely arboroid; walls rough, bristly, and pierced by numerous pores, one (or more) of which is large and conspicuous
Phylum *Porifera*

BB. Body regular in form, walls not pierced by pores and with evidence of a definite symmetry.

C. Anus lacking, mouth capable of opening and closing.

D. Symmetry radial or biradial, radii not five or multiples thereof.

E. Body without rows of ciliated swimming plates; tentacles with batteries of nematocysts (uneven of surface); mouth surrounded by bases of tentacles........Phylum *Cœlenterata*

F. Body vase shaped (tapering toward basal end) with hypostome, with stalk or stem for attachment, and usually colonial; or body umbrella shaped, not colonial.

G. Body vase shaped; or umbrella shaped with velum about margin of umbrella...................Class *Hydrozoa*

GG. Body umbrella shaped without velum and with oral arms; margin of umbrella notched......Class *Scyphozoa*

FF. Body cylindrical, without hypostome, with stomodeum and mesenteries subdividing the gastrovascular cavity; sessile without stalk; single or colonial . . . . Class *Anthozoa*

EE. Body jelly-like in consistency, with eight rows of ciliated swimming plates, tentacles present or absent but without nematocysts. . . . . Phylum *Ctenophora*

DD. Symmetry bilateral, body depressed . . Phylum *Platyhelminthes*

E. Mouth and digestive system present.

F. Without sucking discs about mouth or upon other parts of the body. . . . . Class *Turbellaria*

FF. Sucking discs present. . . . . Class *Trematoda*

EE. Mouth and digestive system absent, body elongate and divisioned into flat units (Roman girdle-like), one end of body undivisioned, narrow with terminal enlargement bearing hooks or adhesive cups or both. . . . . Class *Cestoda*

CC. Anus present, mouth provided with accessory parts such as lobes; palps; hooks, special areas or crowns of cilia; chitinous, calcareous, or bony jaws; teeth, etc.

D. Skeleton absent or external in location.

E. Symmetry radial. Radii usually five or multiple thereof. Surface of body with hard plates and spines or leathery with small calcareous plates. . . . . Phylum *Echinodermata*

EE. Symmetry bilateral, sometimes curved or arranged in a spiral.

F. True metamerism lacking or if the body appears metameric (annulated) it will bear cilia upon the anterior end.

G. Calcareous or horny shells absent, or if present they are tubular or of two valves placed dorsally and ventrally, the ventral valve curved dorsally at narrow end.

H. Body wormlike without crown of tentacles on anterior end.

I. With eversible proboscis which when not everted rests in a sheath above alimentary canal. Proboscis with one or more stylets at end but without hooks Phylum *Nemertinea*

II. Without eversible proboscis. When such a structure is present it is permanently extended and its surface is beset with hooks. Body slender and covered with glistening cuticle. Expanded lips with teeth sometimes present. . . . . Phylum *Nemathelminthes*

HH. Body usually not wormlike but if so the anterior end will bear tentacles.

I. With one or more crowns or girdles of cilia borne upon the discoidal or lobed anterior end. Microscopic in size, not colonial . . . Phylum *Trochelminthes*

II. With an oval or horseshoe-shaped lophophore, bearing tentacles. Calcareous or horny shells may be present as tubular cases or as valves dorsal and ventral in position. Those without shells are colonial, the individuals resting in cases, tubes, or

upon the surface of gelatinous masses
Phylum *Molluscoida*

GG. Calcareous shells present which may be spiral or of two valves lateral in position or segmented and placed on back. If shell appears to be absent the body is elongate with a "saddle-blanket" patch on the back or the head bears a pair of eyes and long tentacles about the mouth each tipped with cuplike adhesive organs
Phylum *Mollusca*

FF. True metamerism obvious.

G. Without jointed locomotor appendages
Phylum *Annelida*

H. Segments bearing parapodia or lateral bristles or both
Class *Chætopoda*

HH. Without either parapodia or bristles.

I. Tentacles present..............Class *Archianellida*

II. Adhesive organ at either end of body, tentacles absent..........................Class *Hirudinea*

GG. Jointed locomotor appendages present
Phylum *Arthropoda*

H. Antennæ present.

I. Antennæ two pairs; gills present....Class *Crustacea*

II. Antennæ one pair.

J. Most body segments bearing similar jointed leg-like appendages. Abdomen not differentiated
Class *Myriapoda*

JJ. Abdomen differentiated and without locomotor appendages. Thorax with three pairs of walking legs. Usually two (sometimes one) pairs of wings present..........................Class *Insecta*

HH. Antennæ absent or apparently so; head and thorax combined, equipped for aerial respiration
Class *Arachnida*

DD. Skeleton internal, metamerism obvious in some, subdued in others. Axial skeleton in form of notochord or vertebral column of cartilage or bone or both. Pharynx with gill slits
Phylum *Chordata*

E. Locomotor appendages absent.

F. Body wormlike with proboscis, collar, and evident gill slits or (in some exotic forms) polyp-like with tentacles at free end. Notochord confined to proboscis
Subphylum *Hemichorda*

FF. Body saclike; inhalant and exhalant apertures; with or without peduncle (stalk), single or colonial in habit
Sybphylum *Urochorda*

EE. Locomotor appendages present in form of mesal or paired fins or both, or as legs.

F. Notochord serving as axial skeleton, continuous mesal finfold present...............Subphylum *Cephalochorda*

FF. Vertebral column and cranium present
Subphylum *Vertebrata*

G. No paired appendages............ Class *Cyclostomata*

GG. Paired appendages present.

H. Paired appendages as fins.

I. Operculum (gill cover) absent Class *Elasmobranchii*

II. Operculum present ....Class *Pisces*

HH. Paired appendages pentadactyle (with digits)

I. Skin naked, no claws on digits......Class *Amphibia*

II. Digits with claws, nails, or hoofs.

J. Horny scales or plates or denticles as body covering........................Class *Reptilia*

JJ. General body covering not in form of scales.

K. Feathers present..................Class *Aves*

KK. Hair present................Class *Mammalia*